VALIDATED UV AND RP-HPLC METHODS FOR THE SIMULTANEOUS ESTIMATION OF AMLODIPINE BESYLATE AND INDAPAMIDE IN BULK AND TABLET DOSAGE FORM

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In partial fulfillment for the award of Degree of

MASTER OF PHARMACY
(Pharmaceutical Analysis)

Submitted by

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MELMARUVATHUR – 603 319

MAY 2012

CERTIFICATE

This is to certify that the research work entitled **"VALIDATED UV AND RP-HPLC METHODS FOR THE SIMULTANEOUS ESTIMATION OF AMLODIPINE BESYLATE AND INDAPAMIDE IN BULK AND TABLET DOSAGE FORM"** submitted to The Tamil Nadu Dr. M.G.R Medical University in partial fulfillment for the award of the Degree of the **MASTER OF PHARMACY** (Pharmaceutical Analysis) was carried out by **C. KUMAR (Register No. 26106125)** in the Department of Pharmaceutical Analysis under my direct guidance and supervision during the academic year **2011-2012.**

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CERTIFICATE

This is to certify that the dissertation entitled **"VALIDATED UV AND RP-HPLC METHODS FOR THE SIMULTANEOUS ESTIMATION OF AMLODIPINE BESYLATE AND INDAPAMIDE IN BULK AND TABLET DOSAGE FORM"** the bonafide research work carried out by **C. KUMAR (Register No. 26106125)** in the Department of Pharmaceutical Analysis, Adhiparasakthi College of Pharmacy, Melmaruvathur which is affiliated to The Tamil Nadu Dr. M.G.R. Medical University under the guidance of **Prof. (Dr.) T. VETRICHELVAN, M. Pharm., Ph.D.**

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Dedicated to

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&

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LIST OF ABBREVIATIONS

%	-	Percentage
% R.S.D	-	Percentage Relative Standard Deviation
µ	-	Micron
µl	-	Microlitre
°C	-	Degree Celsius
AML	-	Amlodipine Besylate
AU	-	Absorption Unit
Gms	-	Grams
HPLC	-	High Performance Liquid Chromatography
IND	-	Indapamide
ICH	-	International Conference on Harmonization
IR	-	Infra Red
LOD	-	Limit of Detection
LOQ	-	Limit of Quantification
mg/ tab	-	Milligram Per Tablet
min	-	Minute
ml	-	Milliliter
ml/ min	-	Milliliter/Minute
ng/ ml	-	Nanogram Per Milliliter
nm	-	Nanometer
pH	-	Negative Logarithm of Hydrogen Ion
Rf	-	Retention factor

rpm	-	Rotations Per Minute
Rt or t_R	-	Retention Time
S.D.	-	Standard Deviation
S.E.	-	Standard Error
USP	-	United States Pharmacopoeia
UV-VIS	-	Ultraviolet – Visible
v/v/v	-	Volume / Volume/ Volume
λ	-	Lambda
µg/ ml	-	Microgram per Millilitre

INTRODUCTION

1. INTRODUCTION

1.1 Analytical chemistry (Kamboj, *et al.*, 2003)

Analytical chemistry deals with various analytical methods for determining and estimating different matters in various states. It is based on the various rings and functional groups present in a molecule. The Analytical chemistry has been split in to two main types

- Quantitative Analysis
- Qualitative Analysis

Quantitative analysis gives the amount of the analyte in the component mixture. Qualitative analysis gives information about the various functional groups and rings in the analyte molecule.

The analytical methods are of two types

- Classical methods
- Instrumental methods

In classical methods, for qualitative analysis, the analyte is extracted and treated with the reagent specific for a functional group to give a coloured reaction. In quantitative analysis, the amount of the analyte in determined by titrimetric method or by gravimetric method.

The instrumental methods are based on the physical properties of the analyte such as the light absorption or emission, conductivity, mass to charge ratio, fluorescence, adsorption and partition etc. The instrumental methods are basically categorized as follows

1. Spectroscopic methods
2. Chromatographic methods
3. Electro analytical methods

4. Thermal methods
5. Light Scattering methods

The instruments used for the instrumental methods basically consist of a source, system under study and the response. The source may be a light source, electrical source or heat source etc., as required by the instrument. The system under study is the analyte under study or any physical character of the analyte. The response is the unit in which the analytical signal from the analyte which is converted to an output signal for the interpretation.

1.2 Analytical Techniques

(Douglas A Skoog, *et al.*, 2004; Willard, *et al.*,1986; Sethi, *et al.*, 2001)

I. Titrimetric methods:

A) Acid – Base Titrations

1. Direct Titrations

a) Titration of an acid by a base

i) Titration of liberated acid

ii) Sorenson – Formol Titration

iii) Non – Aqueous

b) Titration of base by an acid

i) Titration of metal salts

ii) Non – Aqueous

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iv) ICP-MS (Inductively coupled plasma – Mass spectrometry)

Factors Affecting the Choice of Analytical Methods (Mendham, *et al.*, 2002)

- The type of analysis required
- Problem arising from the nature of the material
- Possible interference from components of the material other than those of interest
- The concentration range which needs to be investigated
- The accuracy required
- The facilities available
- The time required for complete analysis
- Similar type of analysis performed

1.3 UV SPECTROSCOPY (Sharma, *et al.,* 2007; Gurudeep R Chatwal, *et al.,* 2009)

The UV spectroscopy is one of the most widely used instrumental analytical techniques for the analysis of pharmaceutical products. The UV region extends from 190 nm to 380 nm. The instrument used to measure the intensity of the UV radiation absorbed or transmitted is known as the ultraviolet - Visible spectrophotometers. A molecule can absorb the UV radiation only when the energy of the radiation similarly to the energy is required to induce electronic transaction in the molecule.

1.3.1 Laws of absorption

When a beam of UV light is allowed to pass through a substance which absorbs the UV light, the intensity of the transmitted light is lesser than the incident light. The reduction of the intensity is may be due to

- Reflections on the surface of the cell
- Scattering of light by macro molecules

Absorption

The two important laws which govern the UV spectroscopy are the Lambert's law and Beer's law. Lambert's law states that the intensity of the light decreases exponentially with decrease in the thickness of the medium through which it passes. Beer's law states that the intensity of the light decreases exponentially with increase in the concentration of the absorbing substance.

The two laws where combined to form the Beer-Lambert's law, which is given by the equation

$$A = abc$$

Where, A is the absorbance

a is the absorptivity

b is the path length

c is the concentration.

The absorptivity is defined as, the absorbance of a substance at a specific wavelength of a 1 g/100 ml solution in a 1cm cell.

1.3.2 Deviation of Beer's law

When the absorbance is plotted against concentration, a straight line passing through the origin should be obtained. But there is always a deviation from the linear relationship in the plot of absorbance versus concentration. The deviation in the Beer's law may be due to anyone of the following reasons.

The presence of foreign substance affects the light absorption and alters the extinction coefficient.

- Due to dissociation or association of the molecule.
- Example, benzyl alcohol in chloroform exists as a polymer. The monomer absorbs at lower wavelength and the polymer at higher wavelength.
- Due to presence of foreign substance which absorbs at the same wavelength as the analyte.
- If monochromatic light is not used.
- Due to undesirable radiations falling on the detector.
- If macromolecules are present. Example, proteins.
- Presence of insoluble particles. Example, suspension.

1.3.3 Transitions in Organic Molecules

(Sharma, *et al.*, 2007; Gurdeep R Chatwal, *et al*., 2009)

The absorption in the ultraviolet region results in the transition of the valence electron form the ground level to the excited level. The three types of electrons involved in the transition are

σ-electrons*:* These are involved in the formation of saturated bonds. The excitation energy for UV radiations is more than electrons in the atom. Hence these electrons do not absorb near UV radiation.

π-electrons: These are involved in the formation of unsaturated bonds. Example: Dienes, trienes and aromatic compounds. It absorbs radiation in near UV region.

n-electrons: These are the lone pair of electrons present in atoms such as oxygen, nitrogen etc., in a molecule. They can be excited by both UV and Visible radiations.

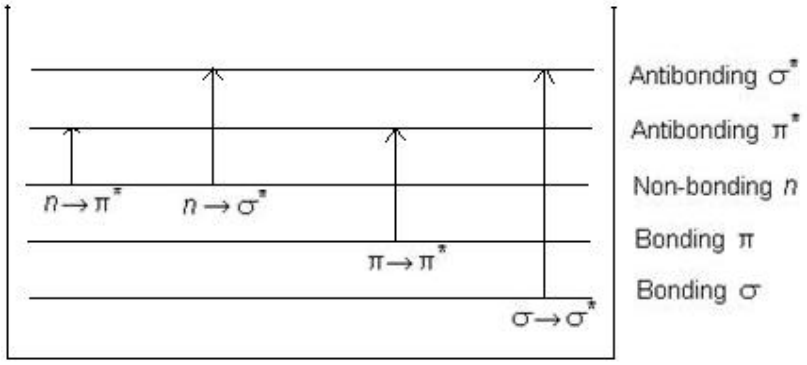
The various types of transitions are

$\sigma \rightarrow \sigma^*$

$n \rightarrow \sigma^*$

$\pi \rightarrow \pi^*$

$n \rightarrow \pi^*$



(http://www.pharmatutor.org/pharma-analysis/analytical-aspects-of-uv-visible-spectroscopy/types-of-electrone-transition.html)

The energy required for the various types of transitions are

$\sigma \rightarrow \sigma^* > n \rightarrow \sigma^* > \pi \rightarrow \pi^* > n \rightarrow \pi^*$

1.3.3.1 σ→σ* transitions

These transitions occur in saturated hydrocarbons with single bonds and no lone pair of electrons. The energy required for this type of transition is very high because of the strong sigma bond formed by the valence electrons. Thus, the transitions occur at very short wavelength. The saturated hydrocarbons such as methane, ethane, propane etc. absorbs at 126 -135 nm region of the UV region. Hence these compounds are used as solvents in UV spectroscopy.

1.3.3.2 n → σ* transitions

Saturated compounds with lone pair of electrons show n→σ* transitions in addition to σ→σ* transitions. The energy required for the n→σ* transition is lesser than the energy required for σ→σ* transitions. The energy required for n→σ* transition, in alkyl halides, decreases with increase in the size of the halogen atom. Alcohols and amines forms hydrogen bonding with the solvent hence require higher energy for the transitions.

1.3.3.3 π → π * transitions

These transitions occur in unsaturated compounds containing double or triple bonds and also in aromatic compounds. Lower energy is required for these transitions and hence a longer wavelength causes the excitation of the molecule.

1.3.3.4 n→ π^* transitions

These transitions occur in compound which contains oxygen, nitrogen, sulphur and halogens because of the presence of free lone pair of electrons. These transitions require least amount of energy and hence they occur in UV and Visible region. Saturated carbonyl compounds shows two types of transitions, low energy n→ π^*

transitions occurring at longer wavelength and high energy n→ π^* transitions occurring at lower wavelength. The shifts in the absorption of the carbonyl compounds are due to the polarity of the solvent.

1.3.4 Transition Probability (Gurudeep R. Chatwal, *et al.*, 2009)

It is not essential that, when a compound is exposed to UV light, transition of the electron should take place. The probability that an electronic transition should take place depends on the value of extinction coefficient. The transitions are classified as

- Allowed transition
- Forbidden transition

1.3.4.1 Allowed transitions

The transitions having ε_{max} value greater than 10^4 are called allowed transitions. They generally arise due to the $\pi \rightarrow \pi^*$ transitions. For example, 1, 3 – butadiene exhibits absorption maximum at 217 nm and has ε_{max} value of 21000 represents allowed transitions.

1.3.4.2 Forbidden transitions

These transitions have ε_{max} value less than 10^4. They occur due to n→ π^* transitions. Example, saturated carbonyl compound (R-C=O) shows absorption near 290 nm and ε_{max} value less than 100 represent forbidden transitions.

1.3.5 Chromophore

These are groups or structure which is responsible to impart colour to the compound. The presence of chromophore is responsible for the absorption of UV radiation by any compound. The groups include nitro group, amine groups, double bonds, triple bonds, etc.

There are two types of chromophore

Groups containing π electrons and undergoes $\pi \rightarrow \pi^*$ transitions.

Example: ethylenes, acetylenes

Groups containing π electrons and n electrons. They undergo two types of transition like $\pi \rightarrow \pi^*$ transitions and $n \rightarrow \pi^*$ transitions.

Example: carbonyls, nitriles, azo compounds etc.

1.3.6 Auxochrome

Any groups which do not itself act as a chromophore but its presence brings a shift in the position of absorption maximum. Chromophores are unsaturated whereas the auxochromes are covalently saturated. The auxochromes are of two types

Co-ordinately unsaturated, example $–NH_2$, -S- groups containing lone pair of electrons.

Co-ordinately saturated, example $–NH_3^+$ groups.

1.3.7 Absorption and Intensity shifts

1.3.7.1 Bathochromic shift or Red shift

The shift in the absorption maximum of a compound, due to the presence of certain auxochromes, towards longer wavelength is called as the bathochromic shift.

1.3.7.2 Hypsochromic shift or Blue shift

The shift in the absorption maximum to shorter wavelength is called Hypsochromic shift. The shift is due to solvent effect or removal of conjugation in a molecule.

1.3.7.3 Hyperchromic effect

The increase in intensity of absorption by inclusion of an auxochrome to a system is hyperchromic shift.

1.3.7.4 Hypochromic shift

The decrease in the intensity of absorption is due to the distortion of the geometry of the molecule.

1.3.8 Solvent effect

The solvent used for the spectral analysis should not interfere in the absorbance of the analyte. It means that the solvent should not have any absorbance in the region under investigation. Based on the polarity of the solvent used the intensity of the absorption changes for a particular analyte. The α, β – unsaturated carbonyl compounds shows two different types of transitions

$$n \rightarrow \pi^{*} \text{ transition}$$

The increase in polarity moves the absorption maximum to a shorter wavelength. The ground state is more polar compared to the excited state.

$$\pi \rightarrow \pi^{*} \text{ transitions}$$

The increase in polarity moves the absorption maximum to longer wavelength. Only lesser energy is required for this transition and hence shows red shift.

1.3.9 Choice of solvent

There are two important requirements a solvent must satisfy to be used as a solvent in UV spectroscopy.

There are

It should be transparent throughout the region of UV under investigation

It should not interact with the solute molecules and should be less polar.

1.3.10 Instrumentation

The components of a UV-Visible spectrophotometer are

- Light source
- Monochromators
- Sample cell
- Detectors

Light source

The various source of light used in the spectrophotometer are tungsten lamp, hydrogen discharge lamp, deuterium lamp, xenon arc lamp and mercury arc lamp. The most commonly used lamp is the deuterium discharge lamp.

Monochromators

The monochromators are used to disperse the light for the required wavelength. The monochromators consists of three units entrance slit, dispersing element and exit slit. The dispersing unit may be filters, prisms or gratings. The fused silica prisms and quartz prisms are commonly used in UV spectrophotometers.

Sample cell

The cell must be transparent throughout the wavelength region of study. The cells are made of fused glass or fused silica or quartz. The glass cells are not used since they absorb in the UV region. Quartz cells are commonly used.

Detectors

The detectors employed are barrier layer cells, photo emissive tubes, photodiodes and photomultiplier tubes. Photodiodes are commonly used in the instrument.

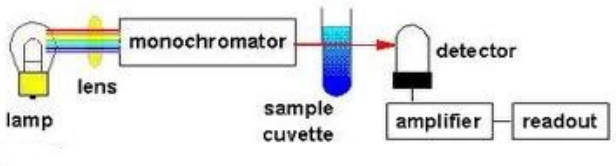
1.3.11 Spectrophotometers

There are two designs of spectrophotometer. They are single beam spectrophotometer and double beam spectrophotometer.

Single beam system

- Light given off from the source
- Lens gathers the light and focuses on the monochromator
- The light of specific wavelength comes out of the monochromator
- Radiation passes through the sample in the sample cell and to the detector

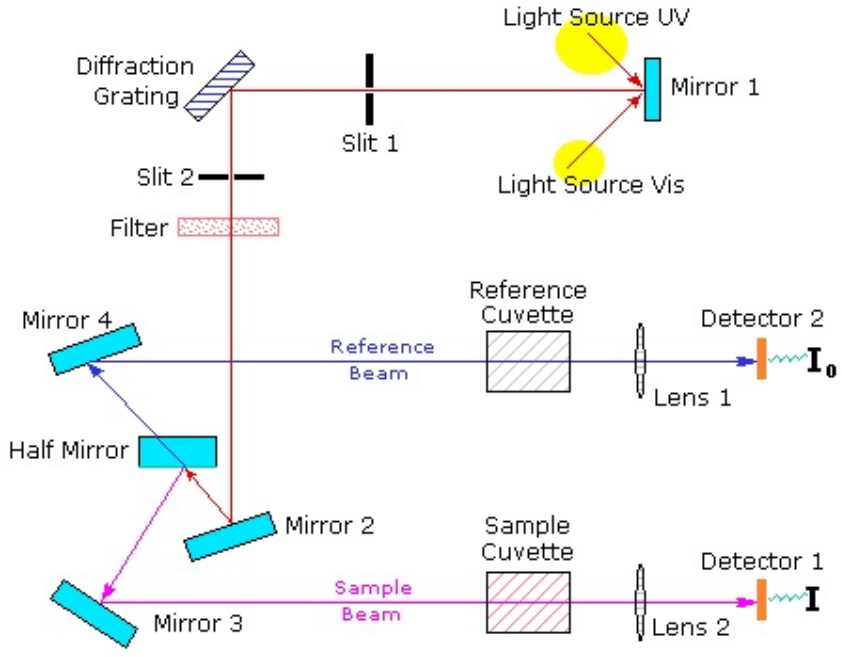
- Detector measures the intensity of the light reaching it.



UV-Visible Single beam spectrophotometer (chemistry.adelaide.edu.au)

Double beam system

- Radiation from source falls on the monochromator
- The radiation of the required wavelength pass out of the exit slit reaches the rotating disc
- Rotating disc splits the beam into two, one passes through the sample cell and the other passes through the reference cell
- The light beam falls on the detector
- Detector measures the intensity of the light.



Double beam UV-Visible spectrophotometer (Chemguide.co.uk)

1.3.12 Quantitative analysis of single component

(Beckett and Stenlake, *et al.,* 2007)

The assay of an analyte is done by dissolving the analyte in a suitable solvent and measuring the absorbance of the solution at the required wavelength. The selected wavelength is the absorbance maximum of the analyte in that particular solvent. The concentration of the analyte can be determined by

- Use of absorptivity value
- Use of calibration graph
- Single or double point standardization

1.3.12.1 Absorptivity value method

This method is usually followed in official books such as Indian Pharmacopoeia, British Pharmacopoeia etc. The advantage of the method is, the preparation of standard solutions of reference substance is not required for the calculation of the concentration of the analyte.

1.3.12.2 Calibration graph method

In this method, a series of linear concentration solutions of the reference solutions are prepared and the value of absorbance is plotted against the concentration of the reference solution. From the graph the absorbance of the sample solution is plotted and the concentration is found.

1.3.12.3 Single point or double point standardization

In single point standardization, the standard and the sample solutions are prepared under same identical condition. Also, the standard and the sample concentration are almost equal. Then after the measurement of absorbance the following formula is applied to find the unknown sample concentration

$$C_{test} = \frac{A_{test}}{A_{std}} \times C_{std}$$

Double point standardization is used when there is a linear but non proportional relationship between concentration and absorbance. The concentration of one of the standard is higher and the concentration of other is lower than that of the standard.

$$C_{test} = \frac{(A_{test} - A_{std1})(C_{std1} - C_{std2}) + C_{std1}(A_{std1} - A_{std2})}{A_{std1} - A_{std2}}$$

1.3.13 Assay of substance in multi component samples

The multi component analysis is done when the sample contains more than one analyte to be quantified in the sample. In such methods one of the analyte may be taken as interferent and the absorbance of the interferent reduced to find the true absorbance of the analyte. Similarly the absorbance of the other analyte is found by taking the first analyte as the interferent.

The determination of the multi-component samples can be done by using the following methods:

- Assay of a single-component sample
- Assay using absorbance corrected for interference
- Assay after solvent extraction of the sample
  - ❖ Simultaneous equations method
  - ❖ Absorbance ratio method (Q-Analysis)
  - ❖ Geometric correction method
  - ❖ Orthogonal polynomial method
  - ❖ Difference spectrophotometry

- ❖ Derivative spectrophotometry
- ❖ Area under curve method
- ❖ Chemical derivatization

Method carried out

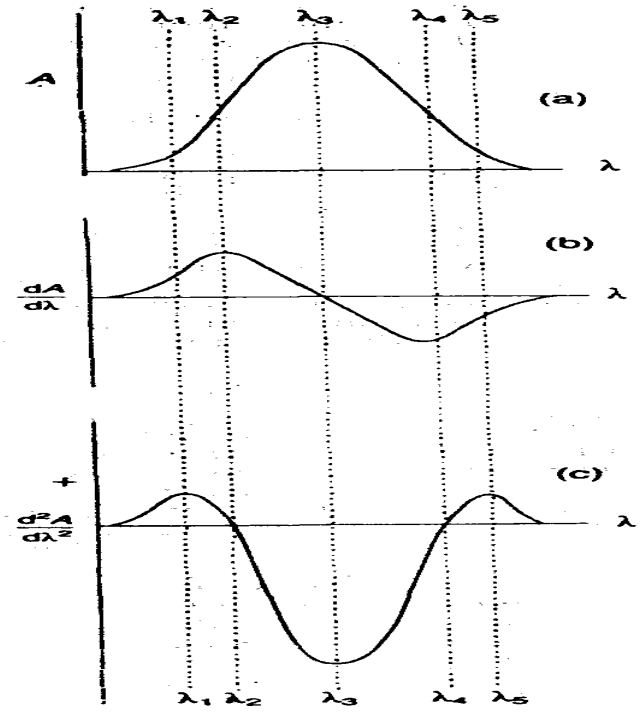
Derivative spectroscopy

Derivative Spectroscopy

Derivative spectrophotometry involves the conversion of a normal spectrum to its first, second or higher derivative spectrum. The transformations that occur in the derivative spectra are understood by reference to a Gaussian band which represents an ideal absorption band. In the context of derivative spectrophotometry, the normal absorption spectrum is referred to as the fundamental, zero order or D° spectrum.

The first derivative (D^1) spectrum is a plot of the ratio of change of absorbance with wavelength against wavelength, i.e. a plot of the slope of the fundamental spectrum against wavelength or a plot of $dA/d\lambda$ Vs λ. At $\lambda 2$ and $\lambda 4$, the maximum positive and maximum negative slope respectively in the D°. Spectrums correspond with maximum and a minimum respectively in the D^1 spectrum. The λ_{max} at λ_3 is a wavelength of zero slopes and gives $dA/d\ \lambda\ 0$, i.e. a cross-over point, in the D1 spectrum.

The second derivative (D^2) spectrum is a plot of the curvature of the D° spectrum against wavelength or a plot of $d^2A/d\lambda^2$ Vs λ. The maximum negative curvature at λ_3 in the D° spectrum gives a minimum in the D^2 spectrum, and at λ_1 and λ_5 the maximum positive curvature in the D° spectrum gives two small maxima called 'satellite' bands in the D^2 spectrum. At λ_2 and λ_4 the



The zeroth (a), first (b) and second (c) derivative spectra of a Gaussian band.

Wavelengths of maximum slope and zero curvature in the D° spectrum correspond with cross-over points in the D^2 spectrum.

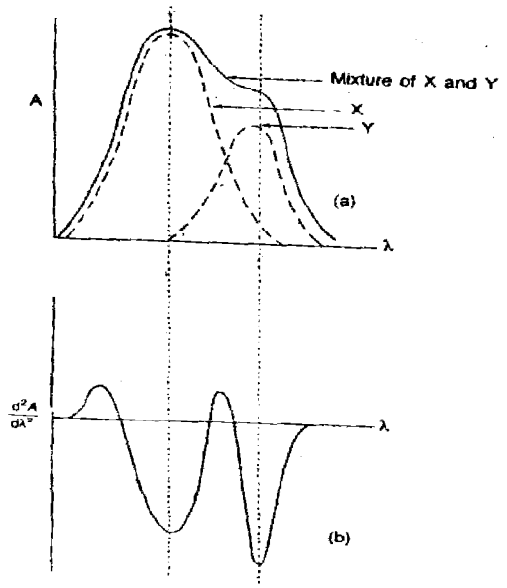
In summary, the first derivative spectrum of an absorption band is characterized by a maximum, a minimum, and a cross-over point at the λ_{max} of the absorption band. The-second derivative spectrum is characterized by two satellite maxima and an inverted band of which the minimum corresponds to the λ_{max} of the fundamental band.

These spectral transformations confer two principal advantages on derivative spectrophotometry. Firstly, an even order spectrum is of narrower spectral bandwidth than its fundamental spectrum. Secondly, derivative spectrophotometry discriminates in favour of substances of narrow spectral bandwidth against broad bandwidth substances. This is because ‘the derivative amplitude (D), i.e. the distance from a

maximum to a minimum, is inversely proportional to the fundamental spectral bandwidth (14') raised to the power (n) of the derivative order. Thus,

$$D \alpha (1/W)^n$$

Consequently, substances of narrow spectral bandwidth display larger derivative amplitudes than those of broad bandwidth substance



(a) The individual spectra of two components X and Y in admixture and their combined spectrum. (b) The second derivative spectrum of the mixture showing improved resolution of the individual bands.

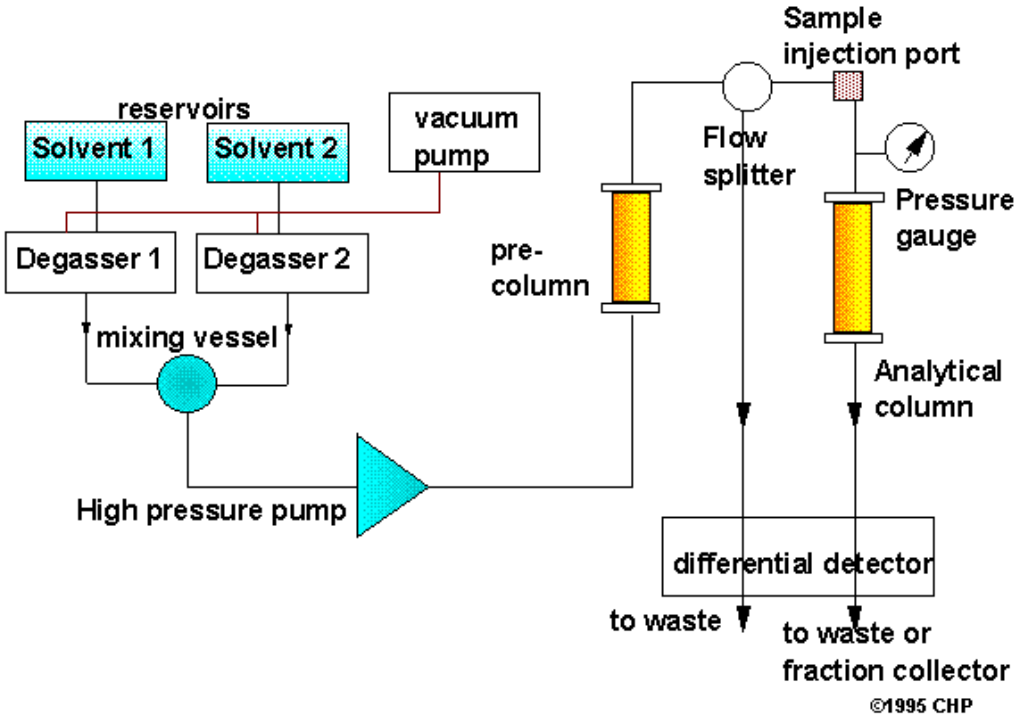
The enhanced resolution and bandwidth discrimination increases with increasing derivative order. However, it is also found that the concomitant increase in electronic noise. Inherent in the generation of the higher order spectra, the consequent reduction of the signal-to-noise ratio, place serious practical limitations on the higher order spectra.

1.4 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

(Sharma, *et al.*, 2000)

The high performance liquid chromatography is thus a method of separation in which the stationary phase is contained in a column one end which is attached to a source of pressurized liquid eluent (mobile phase).

The choice of mobile phase is very important in HPLC and the eluting power of the mobile phase is determined by its overall polarity of the stationary phase and the nature of sample components for normal phase separation eluting power increases with increasing polarity of the solvent ,but for reversed phase separations, eluting power decreases with increasing solvent polarity.



1.4.1. MODES OF SEPARATION (Lloyd, *et al*., 1997)

i. Reversed-phase chromatography

Reversed-phase chromatography (RPC) is the first choice for most regular samples. RPC s typically more convenient and rugged than other forms of liquid chromatography and is more likely to result in a satisfactory final separation. High-performance RPC columns are efficient, stable, and reproducible. Detection often is easier in RPC because of the solvents used. Finally, most workers have more experience with RPC than with other HPLC methods.

Although many organic compounds have limited solubility in the mobile phase, this is not a practical limitation because only small amounts (nanograms or micrograms) of sample are usually injected. In those cases where sample solubility in RPC mobile phases is exceptionally poor, normal-phase chromatography is a preferred alternative. Similarly, samples that are unstable in aqueous media can also be separated by normal phase chromatography using non-aqueous solvents.

ii. Normal-phase chromatography

In normal-phase chromatography (NPC) the stationary phase is more polar than the mobile phase, the opposite of RPC. Usually, the mobile phase is a mixture of organic solvents without added water and the column packing is either an inorganic adsorbent (silica or occasionally alumina) or a polar bonded phase (cyano, diol, or amino) on a silica support. Regardless of the mobile or stationary phase used, sample retention in NPC increases as the polarity of the mobile phase decreases (the opposite of RPC).

NPC has been used for separating both neutral and ionic compounds, but neutral samples predominate. NPC for ionic samples can involve the use of water in

the mobile phase, and the retention process is then somewhat complex. When ionic samples are separated by NPC, it is usually advisable to add triethylamine to the mobile phase for basic compounds and acetic or formic acid for acidic compounds. Neutral samples are often separated equally well by either RPC or NPC, the main difference being a reversal of elution order for the two HPLC methods. In NPC, less polar compounds elute first, while more polar compounds leave the column last: this behavior can be contrasted with the opposite RPC behavior.

iii. Ion-pair chromatography

Ion-pair and reversed-phase HPLC share several features. The column and mobile phase used for these separations are generally similar, differing mainly in the addition of an ion-pair reagent to the mobile phase for ion-pair chromatography (IPC). For most applications that involve ionic samples, RPC separation should be explored first, before considering IPC. IPC separations are more complicated to develop and are subject to additional experimental separation due to poor band spacing, IPC provides an important additional selectivity option. Thus IPC is a logical follow-up for RPC separations that need improvement.

iv. Ion-exchange chromatography

Ion exchange chromatography (IEC) was an important HPLC method. Its application for the separation of most sample types gradually diminished compared to other HPLC methods. Today it is used infrequently, except for certain “special” samples. These include mixtures of biological origin, inorganic salts, and some organometallics.

Because of the similarity of ion-exchange and ion-pair HPLC retention, many separations that are possible using IEC can also be achieved using IPC. For the

separation of typical small-molecule samples, IPC may have certain advantages like higher column efficiencies, easier control over selectivity and resolution, and more stable and reproducible columns.

1.4.2. Elution techniques (Sharma, *et al.,* 2000)

Two types of elution techniques generally used. They are,

i) Isocratic elution

One particle solvent or mixture is pumped through the whole analysis.

ii) Gradient elution

For some determinations the solvent composition may be altered gradually gradient elution system can be classified as low pressure and high pressure system. In low pressure gradient elution system the eluent components are miner proportion varying with time at low pressure and the mixture is pumped in order to be delivered at high pressure to the column. In high pressure gradient elution system components or mixtures of fixed composition are each pumped by separate pump and then mixed at high pressure in a ratio varying with time.

1.4.3. REVERSE-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (Mendham, *et al*., 2002)

High performance systems have meant that liquid chromatography has over taken gas chromatography as HPLC now provide the following features,

1. High Resolving Power
2. Speedy separation
3. Continuous monitoring of the column effluent
4. Accurate quantitative measurement

5. Repetitive and reproducible analysis using some columns

1.4.3.1. Mobile Phase

A successful chromatographic separation depends upon differences in the interaction of the solutes with the mobile phase and the stationary phase. In liquid chromatography, the choice and variation of the mobile phase is of critical importance in achieving optimum efficiency. HPLC grade solvents tend to be costly. To ensure consistent performance, the solvent should not contain any trace amounts of other materials, including water for organic solvents.

1.4.3.2. Pumping Systems (Douglas, *et al*., 2004; Willard, *et al*., 1986)

The requirements for liquid chromatographic pumps include

- Ability to generate pressures of up to 6000 psi (Ibs/in^2)
- Pulse free output
- Flow rate ranging from 0.1 to 10 ml/min
- Flow reproducibility of 0.5% relative or better
- Resistance of corrosion by a variety of solvents

Types of pumps

- Reciprocating Piston Pumps
- Syringe Type Pump
- Constant Pressure Pump

1.4.3.3. Columns for HPLC (Willard, *et al*., 1986)

The columns most commonly used are made from precision bore polished stainless steel tubing; typical dimensions are 10-30 cm long and 4 (or) 5 mm internal diameter. The stationary phase (or) packing is retained at each end by thin stainless steel frits with a mesh of 2 µm or less. The packing used in modern HPLC consist of

small, rigid particles having a narrow particles size distribution. The types of column used in HPLC are

- ❖ Standard columns
- ❖ Radial compression columns
- ❖ Narrow Bore columns
- ❖ Short, fast columns
- ❖ Guard columns and In-line filters

1.4.3.4. Temperature Control

Separation columns should be housed within a stable system with temperature variations of less than 0.1°C, the temperature changes must be avoided. Circulating air bath or electrically heated chambers are used to control the column temperature. The solvent is preheated separately before entering the separation column.

1.4.3.5. Detectors (Ashutoshkar, *et al.*, 2005)

The main function of the detector in HPLC is to monitor the mobile phase coming out the column, which in turn emits electrical signals that are directly proportional to the characteristics either of the solute or the mobile phase.

Basic detector requirements

An ideal LC detector should have the following properties:

1. Low drift and noise level (particularly crucial in trace analysis).
2. High sensitivity
3. Fast response
4. Wide linear dynamic range (this simplifies quantitation)
5. Low dead volume (minimal peak broadening)

Types of detectors

Detector	Types of molecules	General
Refractive Index Detector	Carbohydrates, Polymers	Molecules that do not have a UV chromophore, cannot
UV/Vis, Pho diode array detector	Organic molecules, bio molecules expect carbohydrates	Any molecule that absorbs, light between 170 and 700 nm, PDAs are typically used either when the molecules in the mixture absorb at different wavelengths or when the wavelength of maximum absorption is unknown. PDAs can be used for determining peak
Fluorescence	Aromatic compounds (PAHs) AccQTag,	Generally used for applications that require extremely high sensitivity
ELSD Evaporating light scattering detector	Carbohydrates, Polymers	Molecules that do not have a UV chromospheres, can be used with gradients, generally more sensitive that a RI detector

Mass spectrometer	Organic molecules, bio molecules	Can be used to detect and determine the mass on any molecule that can be ionized and is within the mass range of the specific MS
MS/MS	Organic molecules, bio molecules	Detect and determine the mass of any molecule that can be ionized and is within the mass range of the specific Ms and detailed structural studies to be performed
ECD Electrochemical detector	Carbohydrates, man other organic molecules whose redox potential is different than the mobile	Can provide extra sensitivity and selectivity for molecules that are not readily detected by device
Conductivity	Cations and Anions	Used almost exclusivel for ion chromatography

1.4.4. THEORY OF OPERATION

Column efficiency (Lloyd, *et al.*, 1997; Willard, *et al.*, 1986)

Column efficiency refers to the performance of the stationary phase to accomplish particular separations. Column efficiency is a function of different separation variables. This entails how well the column is packed and its kinetic performance. The efficiency of a column can be measured by several methods which

may or may not be affected by chromatographic anomalies, such as "tailing" or appearance of a "front". This is important because many chromatographic peaks do not appear in the preferred shape of normal Gaussian distribution. For this reason, efficiency can be an enigmatic value since manufacturers may use different methods in determining the efficiency of their columns.

Calculation of column efficiency value

- Inflection method
- Half-peak height method
- Tangent method
- Sigma Method
- Height/Area method
- Moment method

1.4.5. QUANTITATIVE ANALYSIS

Quantification involves the measurement of peak height or peak area. To determine the concentration of a compound, the peak area or height is plotted vs the concentration of the substance. For well resolved peaks, both peak height and area are proportional to the concentration. Four different calibration methods used in quantitative analysis are,

a. Normalized peak area

b. External standard addition method

c. Internal standard addition method

d. Standard addition method

a. Normalized peak area

After completion of a run and the integration of all significant peaks in the chromatogram, the total peak area can be calculated. This technique is widely used to estimate the relative amount of small impurities or degradation compound in a purified material. The area present of any individual peak is referred to as the normalized peak area. The technique of normalized peak area is actually not a calibration method, since there is no comparison to a known amount for any peak in the chromatogram.

b. External standard calibration

The most general method for determining the concentration of an unknown sample is to construct a calibration plot using external standards. Standards are prepared at known concentrations. A fixed volume of each standard solution is Injected and analyzed, and the peak responses are plotted Vs concentration. The standard solutions are referred to as external standards, since they are prepared and analyzed in separate chromatograms from those of the unknown samples. Unknown samples are then prepared, injected and analyzed in exactly the same manner.

c. Internal standard calibration

Another technique for calibration involves the addition of an internal standard to the calibration solutions and samples. The internal standard is a different compound form the analyte, but one that is well resolved in the separation. The internal standard can compensate for changes in sample size or concentration to instrumental variations. With the internal standard method, a calibration plot is produced by preparing and analyzing calibration solutions containing different concentration of the compound of interest with a fixed concentration of the internal added.

The Internal standard comply the following requirements:

- ❖ Well resolved from the compound of interest and other peaks.
- ❖ Similar retention (k) to the analyte.
- ❖ Should not be in the original sample
- ❖ Should mimic the analyte in any sample preparation steps.
- ❖ Does not have to be chemically similar to analyte.
- ❖ Commercially available in high purity.
- ❖ Stable and unreactive with sample or mobile phase.
- ❖ Should have similar detector response to the analyte for the concentration used
- ❖ It must be separated from all compounds of interest in the separation.

d. Method of standard addition

A calibration standard ideally should be prepared in a blank matrix of drug formulation components without the drug substance or an animal without added compound usually can be used for standard calibration solutions. The method of standard addition is most often used in trace analysis. In this approach, different weights of analyte(s) are added to the sample matrix, which initially contains an unknown concentration of the analyte. Extrapolation of a plot of response found for the standard addition calibration concentration to zero concentration defines the original concentration in the unspiked sample.

1.5. ICH Guidelines for Analytical Method Validation Parameters

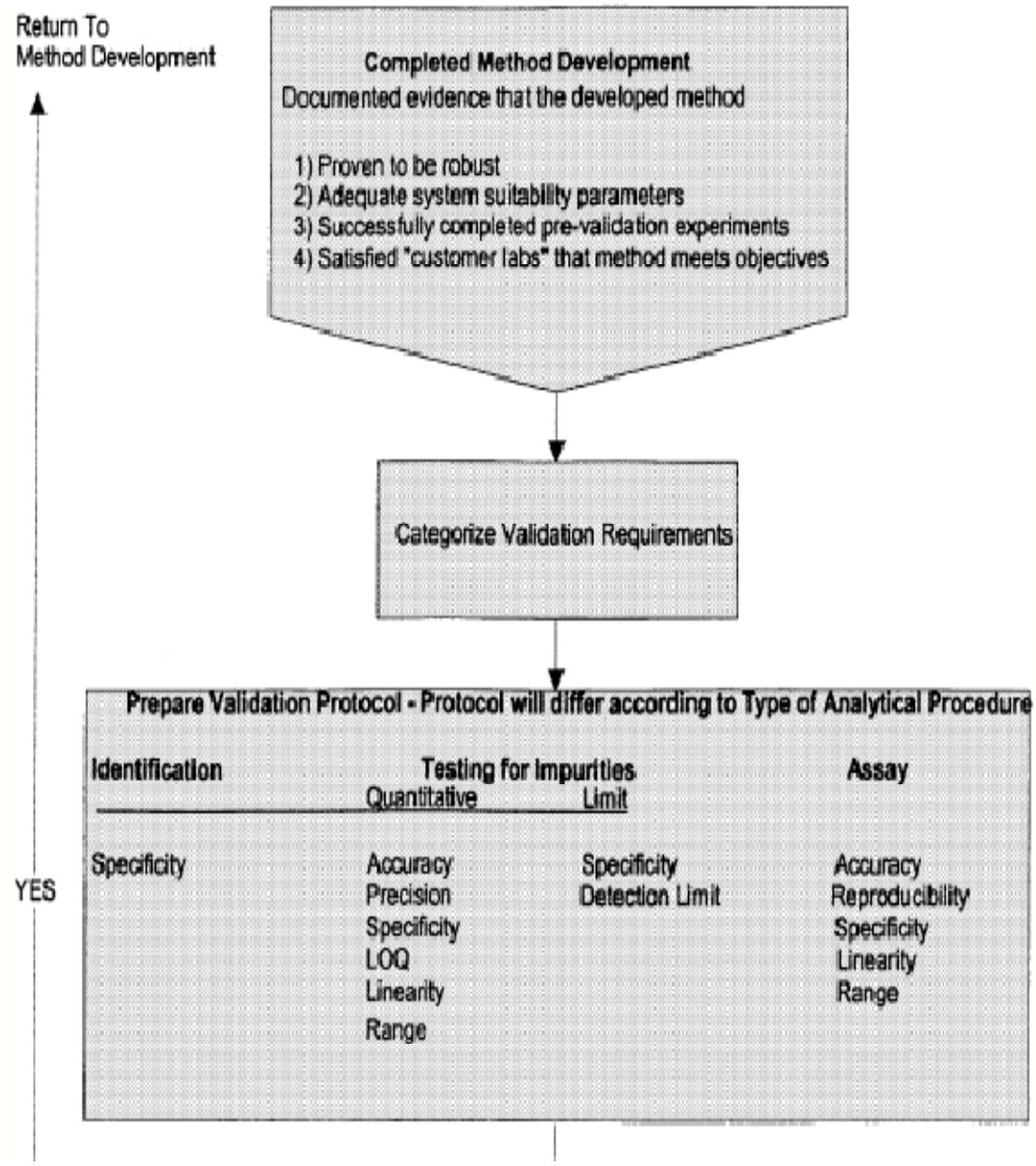
(Code Q2A; Q2B, ICH Guidelines 1994 and 1995)

The analytical methods are validated as per the ICH and USFDA guidelines. The objective of validation of analytical method is to prove that the method is suitable for the specific purpose. Analytical procedure refers to the way of doing an analysis. It describes completely all the steps involved in performing the analysis. The types of analytical procedure to be validated are

- Identification tests
- Quantitative test for impurities
- Limit test for impurities
- Quantitative analysis of the active drug

The various validation parameters specified in the ICH and USFDA guidelines are

- Accuracy
- Precision
- Specificity
- Limit of detection
- Limit of quantification
- Linearity
- Range
- Robustness
- Ruggedness



Accuracy

The word accuracy refers to term trueness. It expresses the closeness between the true value or the reference value and the value found in the analysis. True value is the accepted value of the reference value. The accuracy is determined by the recovery studies.

Precision

Precision expresses the degree of scatter between a series of measurement made in multiple sampling from the same homogenous sample. It may be considered under three levels

- Repeatability
- Intermediate precision
- Reproducibility

The precision is expressed as Variance, standard deviation and coefficient of variation for a series of measurements.

The repeatability is confirmed by a minimum of 6 estimations at 100% of test concentrations. The standard deviations should be less than 2.

The intermediate precision is confirmed by inter day and intraday analysis, different instruments and different analyst.

Specificity

Specificity refers to the ability of the method to assess the analyte in the presence of other components like impurities, matrix or degradants, etc. The implications of specificity are

Identification: to ensure identity

Purity tests: to determine the content of impurity

Assay: content of the analyte in the sample

Limit of Detection

It is the lowest amount of an analyte that can be detected by the analytical procedure but cannot be quantified exactly.

The LOD is performed based on the following parameters

- Based on visual examination

- Based on signal to noise ratio
- Based on the standard deviation and slope value.

The visual examination is done by analysing the sample with known quantity of standard and by establishing the minimum level at which the analyte can be detected.

A signal to noise ratio of 3 o 2.1 is considered as acceptable value for calculating the detection limit. Based on slope and standard deviation values, Detection limit can be calculated by using the formula,

$$LOD = \frac{3.3\sigma}{S}$$

σ = the standard deviation of the response

S = the slope of the calibration curve (of the analyte)

Limit of quantification

The lowest amount of the analyte which can be quantified by an analytical method with precision and accuracy is the limit of quantification.

Three approaches are made for determining the quantification limit. They are similar to that of determining the detection limit.

- Based on visual examination
- Based on signal to noise ratio
- Based on slope and standard deviation value

$$LOQ = \frac{10\sigma}{S}$$

Where

σ = the standard deviation of the response.

S = the slope of the calibration curve (of the analyte).

Linearity

Linearity is the ability of an analytical method to obtain results which are directly proportional to the analyte concentration within a given range. The linearity is evaluated as a plot of signals as a function of analyte concentration. The statistical parameters such as the slope, intercept, regression equation and correlation coefficient are calculated. For establishing of linearity, a minimum of 5 concentrations is required.

Range

It is the interval between the lower and upper limit of concentration in a sample for which the analytical method has suitable precision, accuracy and linearity. The minimum range considered for the assay of drug or finished product is form 80 to 120 percent of the test concentration.

Robustness

It is the ability of an analytical method to remain unaltered by small but deliberate variations in various parameters of the method and indicate its reliability. The typical variation includes stability of analytical solutions and extraction time. In case of HPLC, the change in the ratio of mobile phase, flow rate, variation of pH of the solution are done for determining the robustness of the method.

Ruggedness

The united states of pharmacopoeia (USP) define ruggedness as the degree of reproducibility of test results obtained by the analysis of the same sample under a variety of normal test condition such as different labs, different analysis, different lots of reagents etc. Ruggedness is a measure of reproducibility of test results under normal expected operational conditions from laboratory to laboratory and from analyst to analyst.

Acceptance criteria of validation for HPLC

S.No.	Characteristics	Acceptance Criteria
1	Accuracy	Recovery 98-102% with 80,100,120% spiked sample.
2	Precision	RSD < 2%
2a	Repeatability	RSD < 2%
2b	Intermediate precision	RSD < 2%
3	Specificity/ selectivity	No interference
4	Detection Limit	S/N > 2 or 3
5	Quantitation Limit	S/N > 10
6	Linearity	r = 0.999
7	Range	80-120%
8	Stability	>24 hr or > 2hr

1.6. SYSTEM SUITABILITY

System suitability is the checking of a system to ensure system performance before or during the analysis of unknowns. Before performing any validation experiment, you should establish that the HPLC and the procedure are capable of providing data of acceptable quality. These tests are to verify that the resolution and repeatability of the system are adequate for the analysis to be performed. It is based on the concept that equipment, electronics, analytical operations and sample constitute an integral system that can be evaluated as a whole.

System suitability parameters and recommendations

S.No.	Parameters	Recommendations
1	Theoritical plates (N)	>2000
2	Tailing factor (T)	≤ 2
3	Assymetric factor	≤ 2
3	Resolution (Rs)	> 2 between peak of interest and the closest eluting potential interference
4	Repeatability	RSD ≤ 1% for N ≥5 is desirable
5	Capacity factor (k^1)	> 2.0
6	Relative retention	Not essential as long as the resolution is stated

1.6.1. SYSTEM SUITABILITY PARAMETERS

(Lloyd, *et al.,* 1997; Beckett and Stenlake, *et al.,* 2007)

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations, and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated.

The parameters that are affected by the changes in chromatographic conditions are,

- Column capacity factor (K_A)

- Resolution (R_s)
- Selectivity (α)
- Column efficiency (N)
- Peak asymmetry factor (A_s)
- Tailing factor (T)

1.6.1.1. Column capacity factor (K_A)

The retention of a drug with a given packing material and eluent can be expressed as retention time or retention volume, but both of these are dependent on flow rate, column length and column diameter. The retention is best described as a column capacity ratio (K), which is independent of these factors. The column capacity ratio of a compound (A) is defined as

$$K_A = \frac{V_A - V_0}{V_0} = \frac{t_A - t_0}{t_0}$$

Where,

V_A = Elution volume of A

V_0 = Elution volume of a non retained compound (void volume)

At constant flow rate, retention times (t_A and t_o) can be used instead of retention volumes. Retention data is sometimes expressed, relative to a known internal standard (B). The ratio of retention times (t_A/t_B) can be used, but the ratio of adjusted retention times $\left(\frac{t_A - t_o}{t_B - t_o}\right)$ is better when data need to be transferred between different chromatographs.

The values of k' of individual bands increase or decrease with changes in solvent strength. In reversed phase HPLC, solvent strength increases with the increase in the volume of organic phase in the water / organic mobile phase. Typically an increase in percentage of the organic phase by 10 % by volume will decrease k' of the bands by a factor of 2-3.

1.6.1.2 Resolution (R_s)

The resolution, R_s of two neighboring peaks is defined by the ratio of the distance between the two peak maxima. It is the difference between the retention times of two solutes divided by their average peak width. For baseline separation, the ideal value of R_s is 2.0. It is calculated by using the formula,

$$R_f = \frac{Rt_2 - Rt_1}{0.5\,(W_1 + W_2)}$$

Where,

Rt_1 and Rt_2 are the retention times of components 1 and 2

W_1 and W_2 are peak widths of components 1 and 2.

1.6.1.3. Selectivity (α)

The selectivity (or separation factor), α, is a measure of relative retention of two components in a mixture. The ideal value of selectivity is 2. It can be calculated by using the formula,

$$\alpha = \frac{V_2 - V_0}{V_1 - V_0}$$

Where, V_0 is the void volume of the column and V_2 and V_1 are the retention volumes of the second and the first peak, respectively.

1.6.1.4. Column efficiency

Efficiency, N, of a column is measured by the number of theoretical plates per meter. It is a measure of band spreading of a peak. Smaller the band spread, higher is the number of theoretical plates, indicating good column and system performance. Columns with N ranging from 5,000 to 1,00,000 plates/meter are ideal for a good system. Efficiency is calculated by using the formula,

$$N = 16\frac{Rt^2}{W^2}$$

Where, Rt is the retention time and W is the peak width.

1.6.1.5. Peak asymmetry factor (A_s)

Peak asymmetry factor, A_s can be used as a criterion of column performance. The peak half width b of a peak at 10 % of the peak height, divided by the corresponding front half width a gives the asymmetry factor.

$$A_S = \frac{b}{a}$$

1.6.1.6. Tailing factor (T)

The tailing factor T, a measure of peak symmetry, is unity for perfectly symmetrical peaks and its value increases as tailing becomes more pronounced.

In some cases, values less than unity may be observed. As peak asymmetry increases, integration, and hence precision becomes less reliable.

$$T = \frac{W_{0.09}}{2f}$$

Where,

$W_{0.05}$ = width of peak at 5% height

f = Distance from the peak maximum to the leading edge of the peak, the distance being measured at a point 5% of the peak height from the baseline. Limit: $\leq$ 2 is preferable.

1.6.1.7. Height Equivalent to a Theoretical Plate (HETP)

A theoretical plate can be of any height, which decides the efficiency of separation. If HETP is less the column is more efficient. If HETP is more, the column is less efficient. The height equivalent to a theoretical plate (HETP) is given by

$$\text{HETP} = \frac{\text{Length of the column}}{\text{No. of the theoretical plates}}$$

1.7 BASIC STATISTICAL PARAMETERS (Takeru Higuchi, *et al*., 2001; Gupta, *et al.,* 1994)

In analytical chemistry, statistical methods are unavoidable .whether it is a calibration Curve or the result of single or multi analysis interpretation can only be ascertained if the margin of error is known, when the measurement is repeated, a statistical analysis is compulsory. Total quality control on a statistical basis give promise of making pharmaceutical manufacturing more efficient.

1.7.1 Linear Regression

Once a linear relationship has been shown to have a high probability by the value of the correlation coefficient ‘r’, then the best straight line through the data points has to be estimated. This can often be done by visual inspection of the calibration graph, but in many cases it is far more sensible to evaluate the best straight line by linear regression (the method of least squares). The equation of straight line is

$$y = mx + c$$

Where, y the dependent variable is plotted as result of changing x, the independent variable. To obtain the regression line 'y on x' the slope 'm' of the line and the intercept 'c' on the y axis are given by the following equation.

$$m = \frac{N\Sigma xy - (\Sigma x)(\Sigma y)}{N\Sigma x^2 - \Sigma(x)^2}$$

$$c = \frac{(\Sigma y)(\Sigma x^2) - (\Sigma x)(\Sigma y)}{N\Sigma x^2 - \Sigma(x)^2}$$

1.7.2. Correlation Coefficient

To establish whether there is a linear relationship between two variables x1 and y1**,** use Pearson's correlation coefficient r.

$$r = \frac{n\Sigma x_1 y_1 - \Sigma x_1 y_1}{\{[n\Sigma x_1^2 - (\Sigma x_1)^2][n\Sigma y_1^2 - (\Sigma y_1)^2]\}^{1/2}}$$

Where, n is the number of data points.

The value of r must lie between +1 and -1, the nearer it is to +1, the greater the probability that a definite linear relationship exists between the variables x and y, values close to +1 indicate positive correlation and values close to -1 indicate negative correlation values of 'r' that tend towards zero indicate that x and y are not linearly related (they made be related in a non-linear fashion).

1.7.3. Standard Deviation

It is commonly used in statistics as a measure of precision and is more meaningful than the average deviation. It may be thought of as a root-mean-square

deviation of values from their average and is expressed mathematically as

$$S = \sqrt{\frac{\sum_{i=1}^{i=n} (x_i - \bar{x})_2}{N - 1}}$$

Where SD is Standard deviation

n = Large (50 or more) then of course it is immaterial whether the term in the denomination is n -1 or n

Σ= Summation

x = Mean or arithmetic average

x - $\vec{x}$ = Deviation of a value from the mean

n = Number of observations

1.7.4. Percentage Relative Standard Deviation (%RSD)

It is also known as coefficient of variation. It is defined as the standard deviation (S.D) expressed as the percentage of mean.

$$CV \text{ or } \% \text{ RSD} = \frac{S.D}{\bar{x}} \times 100$$

Where, S D = Standard deviation

$\vec{x}$ = Mean or arithmetic average

The variance is defined as S^2 and is more important in statistics than S itself. However, the latter is much more commonly used with chemical data.

1.7.5. Standard Error of Mean (S.E.)

Standard error of mean can be defined as the value obtained by division of standard deviation by square root of number of observations. It is mathematically expressed as,

$$S.E.=\frac{S.D.}{\sqrt{n}}$$

Where, S D = Standard deviation

n = number of observations

LITERATURE REVIEW

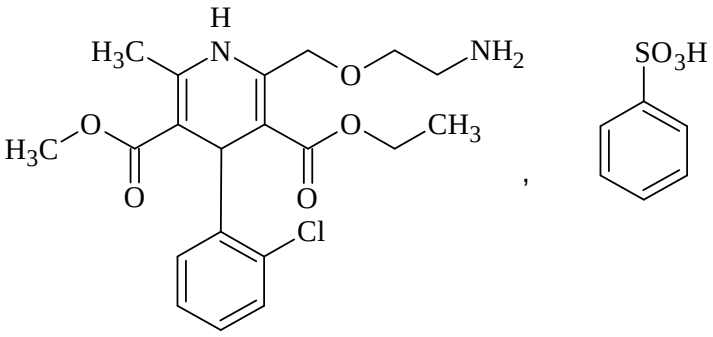
2. LITERATURE REVIEW

2.1 DRUG PROFILE

(The Merck Index, 2006; Indian Pharmacopeia, 2007; The British Pharmacopeia, 2009)

2.1.1AMLODIPINE BESYLATE

Molecular Structure



Chemical Name

3-ethyl 5-methyl2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1, 4-dihydropyridine-3,5-dicarboxylate benzene sulphonate.

Molecular Formula

$C_{26}H_{31}ClN_2O_8S$

Molecular Weight

567.1g/mol

Description

It is a white or almost white powder.

Solubility

Slightly soluble in water, freely soluble in methanol, sparingly soluble in ethanol, slightly soluble in 2-propanol.

Storage

Store protected from light.

pka Value

8.6

Storage

In an airtight container and protected from light.

Purity

98.0 to 102.0

IDENTIFICATION

Melting Point.

Standard Value	Observed Value*
199-201°C	200.3°C

*Average of six determinations

1) **IR Spectrum**

IR spectrum of Amlodipine besylate is compared with the standard Amlodipine besylate IR Spectrum and principal peaks at a wave numbers (KBr disc) were identified. It is shown in Figure 1.

PHARMACOLOGY AND TOXICOLOGY

Category

Amlodipine besylate is a Antihypertensive Agent, Vasodilator Agent, Calcium Channel blockers and Antianginal.

Indications and dosage

Adultzz : Initially 5 mg once daily, increased over 7-14 days.

Elderly : Initially 2.5 mg/day, increased as required

Max Dose : Adult 10 mg/day

Mechanism of action

Amlodipine inhibits the movement of calcium ions (Ca^{2+}) across the cell membrane into vascular smooth muscles and myoctes. Action is greater in the arterial resistant vessels causing peripheral vasodilatation and reduction in after load. Action on the myocardium is considerably less. Clinically, blood pressure is reduced without significant increase in heart rate. In angina patients reduction of after load reduces myocardial oxygen requirement. Severity and frequency of angina attacks are reduced while exercise tolerance is increased.

Pharmacokinetics (Martindale, 2005)

Absorption

Rapidly and completely absorbed with systemic bio-availability of 60 to 65 % in oral dosage form**.**

Distribution

97.5 % bind to plasma protein albumin.

Metabolism

Extensively metabolized in the liver.

Elimination

95 % was eliminated through urine together with less than 10% of a dose of unchanged form.

Protein binding

71-79%

Half-Life

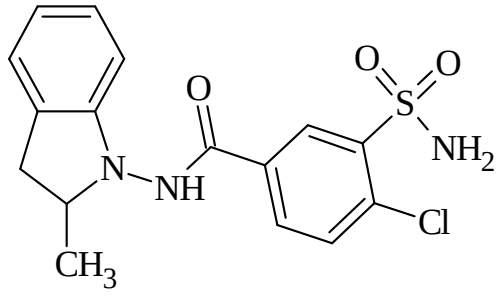
30 hours

Adverse Effect

Peripheral edema, headache, dizziness, lassitude, fatigue, mild hypokalaemia, gingival enlargement, insomnia, tachycardia and hyperglycemia.

2.1.2. INDAPAMIDE

Molecular Structure



Chemical Name

4-chloro-N-[(2RS)-2-methyl-2, 3-dihydro-1H-indol-1-yl]-3-sulphamoylbenzamide.

Molecular Formula

$C_{16}H_{16}ClN_3O_3S$

Molecular Weight

365.84 g/mol

Description

White or almost white powder.

Solubility

Practically insoluble in water, soluble in alcohol and slightly soluble in ether.

Storage

Store protected from light.

pka Value

8.8

Purity

98.0 to 102.0

IDENTIFICATION

1) Melting point

Standard Value	Observed Value*
160-162°C	161.0°C

*Average of six determinations

2) **IR Spectrum**

IR Spectrum of Indapamide is compared with the standard Indapamide IR Spectrum and Principal peak at a wave numbers (KBr disc) were identified. It is shown in Figure 2.

PHARMACOLOGY AND TOXICOLOGY

Category

Antihypertensive Agents and used as Diuretics.

Indications and dosage

Exerts antihypertensive effects at doses lower than those for diuretic action possible relaxation of vascular smooth muscle my inhibition of net Ca2+ inflow.

Adult : Initially 5 mg once daily, increased over 7-14 days.

Elderly : Initially 2.5 mg/day, increased as required

Max Dose : Adult 10 mg/day

Mechanism of Action

Indapamide blocks the slow component of delayed rectifier potassium current (IKs) without altering the rapid component (IKr) or the inward rectifier current. Specifically it blocks or antagonizes the action the proteins KCNQ1 and KCNE1. Indapamide is also thought to stimulate the synthesis of the vasodilator hypertensive prostaglandin PGE2.

Pharmacokinetics (Martindale, 2005)

Absorption

Rapidly and completely absorbed from gastrointestinal tract**.**

Distribution

Strongly bound to red blood cells**.**

Metabolism

Extensively metabolized by hepatic.

Elimination

About 60 to 70% of the dose has been reported to be excreted in the urine; only about 5 to 7% is excreted unchanged. About 16 to 23% of dose is excreted in the faeces.

Protein binding

71-79%

Half-Life

14 hours (bi phasic)

Adverse Effect

Headache, dizziness, electrolyte imbalance (potassium or salt depletion due to too much fluid loss), nausea, weakness.

2.2 REPORTED METHODS

2.2.1 REPORTED METHODS FOR AMLODIPINE BESYLATE

1. **Abdullah Al Masud *et al.* (2011)**

"Validated RP-HPLC method for simultaneous estimation of Amlodipine Besylate and Atorvastatin Calcium".

Stationary phase	:	C18 column (250×4.6 mm, 5 µ
Mobile Phase	:	0.02 M phosphate buffer solution and acetonitrile as (50:50) v/v)
Flow rate	:	1.0 mL /min
Detection	:	PDA detector
Wavelength	:	238 nm.
Retention time	:	AML - 3.5 min ATR - 16.4 min

2. **Vijayavichare *et al.* (2011)**

"Spectrophotometric simultaneous determination of amlodipine besylate and hydrochlorothiazide in combined tablet dosage form by absorption ratio and first order derivative spectroscopy methods".

Solvent	:	Methanol for Method A and method B
Method A	:	Absorption Ratio
Wavelength	:	Amlodipine besylate -271, Hydrochlorothiazide-238.5
Method B	:	First order Derivative Spectroscopy
Wavelength	:	Amlodipine besylate -271, Hydrochlorothiazide-238.5
Linear Range	:	AML: 2.5-25, 2.5-50 µg/ml. HYD: 1-10, 1-20µg/ml.

3. **Devi Ramesh *et al.* (2010)**

"New spectrophotometric methods for simultaneous determination of Amlodipine besylate and Atorvastatin calcium in tablet dosage forms".

Method A	:	Simultaneous equation
Wavelength	:	Atorvastatin calcium at 331 nm and Indapamide 246 nm
Solvent	:	Methanol
Range	:	0.5-30 µg/mL
Method B	:	Q-value analysis
Isoabsorptive point	:	238.8
Solvent	:	Methanol
Range	:	0.5-30 µg/mL

4. **Nilesh Jain *et al.* (2010)**

"RP- HPLC Method for Simultaneous Estimation of Losartan potassium and Amlodipine besylate in Tablet Formulation".

Elution	:	Isocratic elution.
Stationary phase	:	Microsorb C18 Column (5 µm, 250mm x 4.60mm)
Mobile phase	:	methanol– phosphate buffer (pH 4.0) in the ratio of (70:30) % v/v)
Temp	:	isocratic at 25°C ± 0.5°C
Flow rate	:	1.2ml/min
Detector	:	PDA detector

Wavelength	:	248 nm
Retention time	:	AML- 5.59 + 0.5 min LOP- 4.26 + 0.5 min

5. **Kardile *et al.* (2010)**

"Simultaneous estimation of Amlodipine besylate and Olmesartan medoxomil drug formulations by HPLC and UV-spectrophotometric methods".

Elution	:	Isocratic elution
Stationary phase	:	Microsorb C18 Column (5 µm, 250mm x 4.60mm)
Mobile phase	:	0.05 M Pot.dihydrogen phosphate: ACN (50:50 v/v)
Temp	:	isocratic at 25°C ± 0.5°C
Flow rate	:	1.2ml/min
Detector	:	PDA detector
Wavelength	:	230-260 nm
Retention time	:	AML- 3.69 OLM -5.36

6. **Chandan Kumar Giri *et al.* (2010)**

"Simultaneous estimation of nebivolol hydrochloride and amlodipine besylate in combined tablet dosage form by q-analysis method".

Method	:	Q-analysis method
Solvent	:	Methanol
Iso-absorptive point	:	268nm and 282nm

7. **Pournima S patil *et al.* (2011)**

"RP-HPLC method for simultaneous estimation of Amlodipine besylate and olmesartanmedoxomil from tablet".

Solvent	:	Methanol
Method	:	Absorption ratio method.
λmax	:	AML- 238 & 360 nm, NBL-281
Solvent	:	Methanol
Range	:	2-10µg/ml of Indapamide

8. **Chitlange *et al.* (2008)**

"Stability Indicating RP- HPLC Method for Simultaneous Estimation of Valsartan and Amlodipine in Capsule Formulation".

Elution	:	Isocratic elution.
Stationary phase	:	C-18 Column (Kromasil, 250 x 4.6 mm)
Mobile phase	:	Acetonitrile: (0.02M, pH 3.0) phosphate buffer (56:44v/v)
Flow rate	:	1ml/min
Detector	:	UV detector
Wavelength	:	234 nm
Retention time	:	AML and VAT was found to be 3.07 and 6.20 min

9. **Priyanka R Patil *et al.* (2009)**

"RP- HPLC Method for Simultaneous Estimation of Losartan potassium and Amlodipine besylate in Tablet Formulation."

Elution	:	Isocratic elution.
Stationary phase	:	C-18 column
Mobile phase	:	0.02%Triethylamine in water & acetonitrile (60:40)
Flow rate	:	1ml/min
Detector	:	UV detector
Wavelength	:	266 nm
Retention time	:	10.10 min.

10. **Prasad Rao *et al.* (2010)**

"HPLC method for quantization of Amlodipine besylate and Metoprolol Succinate from bulk drug and pharmaceutical formulations".

Elution	:	Isocratic
Stationary phase	:	Inertsil ODS-CV column
Mobile phase	:	0.02 M phosphate buffer solution and acetonitrile as (80:20)
Flow rate	:	1 ml/min.
Detector	:	PDA detector
Wavelength	:	215 nm.

2.2.2 REPORTED METHODS FOR INDAPAMIDE

1. Pawar e*t al.* (2011)

"Quantitative Estimation of Indapamide by Ultraviolet Spectrophotometric Method".

Solvent : Methanol

Method : Simultaneous estimation.

λmax : 240 nm

Solvent : Methanol

Range : 2-10µg/ml of Indapamide.

2. Gaikwad *et al.* (2010)

"Development and validation of UV-Spectrophotometric Method for simultaneous estimation of atenolol and Indapamide in bulk and tablet dosage form".

Wavelength : Atenolol at 225 nm and Indapamide 240 nm

Solvent : Methanol

Range : 6-30 µg/ml for ATN and 2-10 µg/ml for IND

3. Patel amit *et al.* (2011)

"Method development and validation of simultaneous estimation of Telmisartan and Indapamide by reverse phase-high performance liquid chromatography in pharmaceutical dosage forms".

Elution	:	Isocratic
Stationary phase	:	Amazon C18, 5micron, 150 x 4.6 mm
Mobile phase	:	KH_2po_4: Acetonitrile: Methanol (45:25:30v/v/v)
Flow rate	:	1 ml/min.
Detector	:	UV detector
Wavelength	:	285 nm
Retention time	:	For Indapamide 4.7 minutes and Telmisartan 10.7 minutes.

4. Savita S Yadav *et al. (*2011)

"Simultaneous HPTLC analysis of atenolol and indapamide in tablet formulation".

Stationary phase	:	aluminum foil plates precoated with silica gel 60F254
Mobile phase	:	Toluene: ethyl acetate: methanol: (ammonia 5:3:3:0.1 v/v)
Wavelength	:	229nm
Rf Value	:	Indapamide: 0.70, Atenolol: 0.27

5. Pawar Prachi Vasant *et al.* (2011)

"Development and Validation of RP-HPLC method for simultaneous estimation of Atenolol and Indapamide in pharmaceutical dosage form".

Stationary phase	:	Amazon C18, 5micron, 200 x 4.6 mm
Mobile phase	:	Methanol: Water: Diethylamine: Glacial acetic acid (70:30:0.12:0.08v/v/v/v)
Flow rate	:	1.2 ml/minute

Wavelength : 240nm

Retention time : Indapamide: 2.758, Atenolol: 1.858

6. Mohit G Dewani *et al.* (2011)

"Simultaneous estimation of Perindopril Erbumine and Indapamide in bulk drug and tablet dosage form by HPTLC".

Stationary phase : Silica gel 60 F254.

Mobile phase : Dichloromethane: Methanol : Glacial acetic acid in the ratio of (9.5:0.5:0.1 v/v/v.)

Wavelength : 215nm

Rf Value : Indapamide: 0.50 ± 0.02, Atenolol: 0.30 ± 0.02

7. Darshana K Modi *et al.* (2010)

"Development and validation of spectrophotometric method for simultaneous estimation of Perindopril and Indapamide in combined dosage form by Absorbance correction method".

Solvent : Methanol

Method : Absorbance correction method.

λmax : Perindopril- 210.4nm and Indapamide - 285.8nm

Solvent : Methanol

Range : 24 – 56 µg mL-1 of Perindopril 7.5 – 17.5 µg mL-1of Indapamide.

8. Tushar G Barot *et al.* (2009)

"Validated RP-HPLC methods for simultaneous estimation of Indapamide impurity (Methyl Nitrosoindoline) API form".

Elution	:	Isocratic
Stationary phase	:	Inertsil ODS 3V, (150 x 4.6 mm, 5µm)
Mobile phase	:	Triethyl amine pH 2.8: Acetonitrile: Tetrahydrofuran (73:7:20 v/v/v)
Flow rate	:	1.4 ml/min.
Detector	:	PDA detector
Wavelength	:	305 nm
Retention time	:	For Indapamide 16.93 minutes and Methyl Nitrosoindoline 14.13 minutes.

9. Tulja rani *et al.* (2011)

"Validated RP-HPLC Method for simultaneous estimation of Atenolol and Indapamide in pharmaceutical formulations".

Elution	:	Isocratic
Stationary phase	:	C18 column (250×4.6 mm, 5 µ)
Mobile Phase	:	Methanol and water (adjusted to pH 2.7 with 1% orthophosphoric acid) in the ratio of (80:20 v/v)
Flow rate	:	1.0 mL /min
Detection	:	UV-Visible detector
Wavelength	:	230 nm.

10. Jyoti Pai *et al.* (2011)

"Development and Validation of RP-HPLC method for Quantitative estimation of Indapamide in Bulk and Pharmaceutical dosage forms".

Elution	:	Isocratic
Stationary phase	:	C-18 Column (25cm x 4.6 mm i.d.,particle size 5 μm)
Mobile phase	:	Acetonitrile:o-phosphoric acid (0.05%) buffer of pH 3.0 (60:40v/v)
Flow rate	:	1 ml/min.
Detector	:	PDA detector
Wavelength	:	240 nm
Retention time	:	For Indapamide 6.76 minutes.

AIM AND PLAN OF WORK

3. AIM AND PLAN OF WORK

3.1 AIM OF WORK

The prime importance of drug analysis is to gain information about the qualitative and quantitative compositions of substance and chemical species, that is to find out what a substance is composed of and exactly how much.

This information guides development of the manufacturing operations and therapeutic action of drugs.

Standard analytical procedure for newer drugs or its formulation may not be available in Pharmacopoeias. Hence it is essential to develop newer analytical methods which are accurate, precise, specific, linear, simple and rapid.

Amlodipine besylate and Indapamide is a newer combination which is yet to be launched in Indian market.

Hence, the present study aims to develop simple, precise and accurate methods for the determination of Amlodipine besylate and Indapamide by simple UV methods and RP-HPLC method.

3.2 PLAN OF WORK

The combination dosage form selected for the present study contains Amlodipine besylate and Indapamide in solid oral dosage forms, recently this combination of the drugs are introduced into the market.

Method Development

The solubility of the individual drugs was checked from the list of solvents present in Indian Pharmacopeia and the common solvents for the drugs are selected.

The solvent for both UV spectroscopy and RP-HPLC must be less cost and readily available.

UV Spectroscopy

- Selection of appropriate wavelength and selection of suitable method
- Determination of working concentration range
- Analysis of synthetic mixture
- Simultaneous analysis of the formulation by using the developed method

RP-HPLC

- Determination of suitable mobile phase
- Determination of Stability
- Determination of suitable detection wavelength by using UV/VIS and PDA Detector
- Optimization of chromatographic conditions
- Analysis of formulation
- System suitability testing

VALIDATION

The method to be developed should be validated as per ICH guidelines. The various parameters of validation are Accuracy, Precision, Linearity, Range, Repeatability, Limit of detection, Limit of Quantification, and Ruggedness.

MATERIALS AND METHODS

4.0 MATERIALS AND METHODS

4.1MATERIAL USED

DRUGS

Amlodipine besylate was generously gifted by Aurbindo Pharmaceuticals Hyderabad and Indapamide was generously gifted by Micro labs Ltd., Bangalore.

FORMULATION USED

The formulation NATRILAM containing Amlodipine besylate equivalent to 5 mg of Amlodipine and 1.5 mg of Indapamide was procured from a local Pharmacy.

CHEMICAL AND SOLVENTS USED

Methanol (HPLC grade)

Acetonitrile (HPLC grade)

Water (HPLC grade)

Glacial Acetic acid (Analytical grade)

Methanol (Analytical grade) and

Boric acid (Analytical grade)

Sodium hydroxide (Analytical grade)

INSTRUMENTS USED

Different instruments used to carry out the present work.

Shimadzu AUX-220 Digital balance

Mettler Toledo AB204-S/FACT

Shimadzu- 1700 Double Beam- UV- Visible spectrophotometer

Alliance Waters E2695 modular HPLC System

Waters 2489 UV/Visible Detector

Waters 2489 PDA Detector

Sonorex Bendelin Digital 10p apparatus

Micropipette

Elico LI 127 pH meter

Milli-Q-Millipore Integral 3

Specifications (Terms) of Instruments

Shimadzu AUX- 220 Digital Balance (Shimadzu Instruction Manual)

SPECIFICATIONS	
Weighing capacity	200 gms
Minimum display	0.1 mg
Standard deviation	$\leq$ 0.1 mg
Operation temperature range	5 to 40°C

Double Beam UV- Visible Spectrophotometer (Shimadzu and ELICO Instruction Manuals) Model:

- Shimadzu UV-1700; Double beam UV-Visible Spectrophotometer
- ELICO SL – 210; Double beam UV-Visible Spectrophotometer

Specification	Shimadzu UV-1700	Elico SL -210
Light source	20 W halogen lamp, Deuterium lamp, Light source position automatic adjustment mechanism. Built in lamp lighting time display function.	Tungsten halogen lamp (W), Deuterium lamp (D), Light source position automatic adjustment mechanism.
Monochromator	Aberration- correcting concave blazed holographic grating.	Concave holographic grating with 1200 lines/ mm
Detector	Silicon photodiode	Photodiode
Stray Light	0.04% or less (220 nm; NAI 10g/lt) 0.04% or less (340 nm; $NaNo_2$ 50g/lt).	<0.05% T at 220 nm with NAI 10g/ lt
Measurement Wavelength range	190 ~ 1100 nm	190 ~1100 nm
Spectral Band width	1 nm or less (190 to 900 nm).	1.8 nm
Wave length Accuracy	± 0.5 nm on broad automatic wavelength calibration mechanism.	± 0.5 nm automatic wavelength calibration mechanism.

Recording range	Absorbance; - 3.99 ~3.99 Abs Transmittance; - 399 ~ 399%	Absorbance; ± 3.000 Abs
Photometric accuracy	± 0.004 Abs (at 1.0 Abs). ± 0.002 Abs (at 0.5 Abs).	0.005 Abs (at 1.0 Abs). 0.010 Abs (at 0.5 Abs).
Operating Temperature/ Humidity	Temperature range; 15 to 35˚C Humidity range; 35 to 80% (15 to below 35˚C) 35 to 70% (30 to below 35˚C)	Temperature range; 15 to 35˚C Humidity range; 35 to 80% (15 to below 35˚C) 35 to 70% (30 to below 35˚C)

Methods

In the present work an attempt was made to develop and validate a simple, precise and accurate method for the estimation of Amlodipine Besylate and Indapamide in pure and in combined tablet dosage form by UV-Spectrophotometry and HPLC method.

4.2.1 UV-SPECTROSCOPY

Derivative Specrophotometric Method

A simple, accurate, rapid and precise, First order derivative Spectrophotometric method was developed and validated. The first derivative spectrum is a plot of the rate of change of absorbance with wavelength against wavelength, i.e. a plot of the slope of the fundamental spectrum against wavelength.

Selection of solvent

The solubility of Amlodipine besylate and Indapamide was determined in variety of solvents as per Indian Pharmacopoeia standards. Solubility was carried out in polar and non-polar solvents. The common solvent was found to be methanol followed by 0.2M Borate buffer - pH 8.0 is chosen as the best and economic solvent for the analysis of Amlodipine besylate and Indapamide for the proposed methods. From the solubility studies, Methanol followed by dilution with 0.2M Borate buffer (pH-8.0) were chosen as solvent for Spectrophotometry, and it was selected on account of its ready availability, cost factor, solubility, stability factor and cutoff wavelength.

Preparation of 0.2M Borate buffer – pH 8.0

3.09 gm of Boric acid and 3.72 gm of Potassium chloride were dissolved in 500 ml of water, adjusted to pH 8.0 with 0.2M Sodium hydroxide (about 3.9 ml) and diluted with water to 1000 ml.

Preparation of Amlodipine besylate standard stock solution

Weighed accurately about 25 mg of Amlodipine besylate and transferred into a 100 mL volumetric flask separately, dissolved in methanol and made up to the volume with methanol. These solutions were observed to contain 250 mcg/ml of Amlodipine.

Preparation of Indapamide standard stock solution

Weighed accurately about 7.5 mg of Indapamide and transferred into a 100 mL volumetric flask separately, dissolved in methanol and made up to the volume with methanol. These solutions were observed to contain 75 mcg/ml of Indapamide.

Selection of Wavelength

The standard stock solutions were further diluted with 0.2M Borate buffer-pH 8.0 and obtained 10 µg/ml. The solution was scanned between 200 and 400 nm ranges using methanol followed by Borate buffer. For derivative Spectroscopic method, the zero order spectrums was derivatised to first order and the wavelength 339.0 was selected for the estimation of Amlodipine besylate which is zero crossing for Indapamide where as 293.0 nm was selected for the estimation of Indapamide which is zero crossing for Amlodipine besylate. The calibration curve for all the wavelengths were constructed by plotting absorbance Vs concentration for simultaneous equation and $\Delta A/\Delta\lambda$ for first order derivative method. The stability was performed by measuring the absorbance of same solution at different time intervals. It was found that Amlodipine besylate and Indapamide were stable for more than 3 hours.

Stability studies

The Stability studies were performed by measuring the absorbance of same solution at different time intervals. It was observed that Amlodipine besylate and Indapamide in the specified solvent were stable for more than 3 hours at their wavelength maxima.

Preparation of calibration graph

For Amlodipine besylate

132.10 Equivalent of Amlodipine besylate working standard were weighed and transferred into 25 ml volumetric flasks separately. Dissolved in Methanol and made up to the volume with methanol (3750 µg/ml). 1ml of the solution was transferred into a 25 ml volumetric flask and made up to the required volume with 0.2M Borate buffer pH 8.0 to get the concentration 150 µg/ml. 1–6 ml were transferred into a series of 10

ml volumetric flasks and made up to the mark with Borate buffer. The absorbance of different concentration solutions was measured at 339.0 nm against blank. The calibration curve was plotted using concentration against absorbance. The solutions were found to be linear with the concentration range of 15 – 90 µg/ml. The procedure was repeated for three times.

For Indapamide

31.25 mg of Indapamide raw material was weighed and transferred into 25 ml volumetric flask. Dissolved in methanol and made up to the volume with methanol (1250 µg/ml). 1ml of the solution was transferred into a 25 ml volumetric flask and made up to the required volume with 0.2M Borate buffer–pH 8.0 to get the concentration 50 µg/ml. From the aliquots of stock solution of Indapamide 1–6 ml were transferred into a series of 10 ml volumetric flasks and made up to the mark with Borate buffer. The absorbance of different concentration solutions was measured at 293.0 against blank. The calibration curve was plotted using concentration against absorbance. The solutions were found to be linear with the concentration range of 5 – 30 µg/ml. The procedure was repeated for three times.

Quantification of formulation

Twenty tablets of (NATRILAM containing Amlodipine besylate 5 mg of Amlodipine and 1.5 mg of Indapamide, were weighed accurately. The average weight of tablets was found and powdered. The tablet powder equivalent to 25 mg of Amlodipine was weighed transferred into a 100 ml volumetric flask and added a minimum quantity of Methanol to dissolve the substance and made up to the volume with the same (250 µg/ml). The solution was sonicated for 15 minutes, centrifuged for 15 minutes at 100 rpm and filtered through Whatmann filter paper No. 41. From the clear solution, further dilutions were made by diluting 1 ml of the solution into a 100

ml volumetric flask and made up to the required volume with 0.2M Borate buffer to get the concentration of 25 µg/ml of Amlodipine besylate 7.5 µg/ml of Indapamide. The absorbance measurements were made six times for the formulation at 339.0 nm and 293.0 nm. Amlodipine Besylate and Indapamide were found by constructing First order derivative spectrophotometric method.

Recovery studies

The recovery experiment was performed by adding known concentrations of Amlodipine besylate and Indapamide raw material to the 50% preanalyzed formulation. Standard Amlodipine besylate and Indapamide in the range of 15 µg/ml, 22.5 µg/ml, 30 µg/ml, 37.5 µg/ml, 45 µg/ml, 52.5 µg/ml and 5 µg/ml, 7.5 µg/ml, 10 µg/ml, 12.5 µg/ml, 15 µg/ml, 17.5µg/ml was added to the 50% preanalyzed formulation into a series of 10 ml volumetric flasks, diluted with Borate buffer and made up to the mark with the same solvent. The contents were sonicated for 15 minutes. After sonication, the solutions were filtered through Whatmann filter paper No. 41. The absorbance of the resulting solutions was measured at their selected wavelengths for the determination of Amlodipine besylate and Indapamide respectively. The amount of each drug recovered from the formulations was calculated by using first order derivative spectrophotometric method. The procedure was repeated for three times for each concentration.

Validation of developed method

Linearity

A calibration curve was plotted between concentration and absorbance. Amlodipine besylate was linear with the concentration range of 15 – 90 µg/ml at 339.0 nm and Indapamide showed the linearity in the range of 5 – 30 µg/ml at 293.0 nm.

Accuracy

Accuracy of the method was confirmed by recovery studies. To the pre analyzed formulation, a known quantity of working standard of Amlodipine besylate and Indapamide were added and the procedure was followed as per the analysis of formulations. The amount of each drug recovered was calculated. This procedure was repeated for three times for each concentration. The % RSD was calculated.

Precision

The repeatability of the method was confirmed by the analysis of formulations and repeated for six times with the same concentration. The amount of each drug present in the tablet formulations was calculated. The percentage RSD was calculated. The intermediate precision of the method was confirmed by intraday and inter-day analysis i.e. the analysis of formulations was repeated three times in the same day and one time three successive days. The amount of drugs was determined and percentage RSD also calculated.

Ruggedness

Ruggedness of the method was confirmed by the analysis of formulations by using the different analysts with the different instruments. The amount and % RSD were calculated.

Limit of detection (LOD) and Limit of Quantification (LOQ)

Preparation of calibration curve from the serial dilutions of standard was repeated for six times. The Limit of detection and limit of quantification was calculated by using the average value of slope and standard deviation of the intercept.

4.2.2 RP-HPLC METHOD DEVELOPMENT AND OPTIMIZATION OF CHROMATOGRAPHIC CONDITIONS

Selection of chromatographic method

Proper selection of the method depends upon the nature of sample, polarity, molecular weight, Pka value and solubility. The drugs Amlodipine besylate and Indapamide for the present study were polar. So, Reverse Phase Chromatographic technique was selected by using C_{18} column as a stationary phase with different mobile phase.

Selection of mobile phase and wavelength

Different mixtures of mobile phase with different ratios were selected and their chromatograms were recorded. As a result Acetonitrile: Methanol: 0.04M Phosphate buffer-pH 3.0 (25:30:45 v/v/v) was selected as mobile phase. The two drugs were eluted with sharp peak and with better resolution while using the above ratio. Hence this mobile phase was used to optimize the chromatographic conditions.

And scanned in the UV region of 200 – 400 nm and recorded the spectrums. It was found that two drugs have marked absorbance at 240.0 nm and can be effectively used for the estimation of two drugs without interference. Therefore 240.0 nm was selected as detection wavelength for estimation of two drugs by RP – HPLC method with an Isocratic elution technique.

Stability of sample solutions

Solutions of Amlodipine besylate and Indapamide (10 µg/ml) absorbance were checked for their stability at 240 nm and it was found that two drugs were stable for three hours.

OPTIMIZATION OF CHROMATOGRAPHIC CONDITIONS

Initial separation conditions

The following chromatographic conditions were preset initially to get better resolution of Amlodipine besylate and Indapamide.

Mode of operation	-	Isocratic
Stationary phase	-	C_{18} column (250 mm x5.6 mm i.d. 5µm)
Mobile phase	-	Acetonitrile: Methanol: Phosphate buffer - pH 3.0
Proportion of mobile phase	-	30:40:30 % v/v/v
Detection wavelength	-	240 nm
Flow rate	-	1.0 mL/min
Temperature	-	Ambient
Sample load	-	20 µL
Operating pressure	-	190 kgf
Method	-	External Standard Calibration method

The mobile phase was primarily allowed to run for 20 minutes to record a steady baseline. Mixture of Solutions of Amlodipine besylate and

Indapamide were injected and the respective chromatogram was recorded. It was found that both Amlodipine besylate and Indapamide were spitted, for these reason different ratios of mobile phase with different solvents were tried to obtain good chromatogram with acceptable system suitability parameters.

Selection of mobile phase

A mobile phase contains Acetonitrile: Methanol: (0.04M) Phosphate buffer pH 3.0 was selected.

The ratio of the mobile phase is

S. No	Mobile phase	Observation
1	Acetonitrile: Methanol: (0.04M) Phosphate buffer-pH 3.0 (25:30:45 v/v/v)	Both the Amlodipine besylate and Indapamide were eluted with sharp peak

Optimized chromatographic conditions

The following optimized conditions were employed for analysis Amlodipine besylate and Indapamide by Isocratic RP – HPLC method.

Mode of operation - Isocratic

Stationary phase - C_{18} column (250 mm x 5.6 mm i.d. 5µm)

Mobile phase - Acetonitrile: Methanol: Phosphate buffer - pH 3.0

Diluent - Methanol

Proportion of mobile phase	-	25:30:45 % v/v/v
Detection wavelength	-	240 nm
Flow rate	-	1.0 mL/min
Temperature	-	Ambient
Sample load	-	20 µL
Operating pressure	-	121kgf
Method	-	External Standard Calibration method

Preparation of Mobile phase

Preparation of standard stock solution

100 mg and 30 mg of Amlodipine Besylate and Indapamide were weighed accurately and transfer in to a 100 ml standard flask. The volume was made up to mark with methanol to get 1000 µg/ ml and 300 µg/ml of Amlodipine besylate and Indapamide respectively.

Preparation of Calibration graph

From the above stock solution, 8-12 ml was transferred into the series of five 100 ml volumetric flask and made upto the volume with mobile phase to get the concentration 80-120 µg/ml of Amlodipine besylate and 24-36 µg/ml of Indapamide. The solutions were injected and the chromatograms were recorded at 240.0 nm. The above concentration range was found to be linear and obeys Beer's law. The procedure was repeated for six times. The peak areas were plotted against concentration and the calibration curve was constructed.

Estimation of Amlodipine besylate and Indapamide in tablet formulation

Estimation of Amlodipine besylate and Indapamide in tablet formulation by RP – HPLC was carried out using optimized chromatographic conditions. Twenty tablets of formulation (Containing Amlodipine besylate equivalent to Amlodipine 5mg and Indapamide 1.5 mg) were weighed accurately. The average weight of tablets was found and powdered. The tablet powder equivalent to 25 mg of Amlodipine was weighed and transferred into a 50 mL volumetric flask and added a minimum quantity of methanol to dissolve the substance and the content was sonicated for 15 minutes, and made up to the volume with methanol then filtered through Whatmann filter paper No. 41. From the clear solution, the dilutions were made by diluting 10 mL into 50 mL with mobile phase and to get the concentration of 100 µg/ml of Amlodipine and 30 µg/ml of Indapamide theoretically. This solution is used for further analysis.

Assay Procedure

From the above stock solution, 10 mL was pipetted into six different 100 mL volumetric flasks and made up the volume with mobile phase to obtain 100 µg/ml solution of Amlodipine and 30 µg/ml of Indapamide. A steady base line was recorded with optimized chromatographic conditions. After the stabilization of base line for 20 minutes, six test solutions of formulation were injected and recorded the chromatograms. The concentration of each test solution was determined by using slope and intercept values from the calibration graph.

Recovery Experiments

a) Preparation of Amlodipine besylate and Indapamide raw material stock solution

An accurately weighed quantity of 100 mg of Amlodipine besylate and 30 mg of Indapamide were transferred in to a 100 mL volumetric flask and added sufficient methanol to dissolve the substance and made up to the mark with the same solvent. The above solutions were further diluted to get final concentration of 1000 µg/ml of Amlodipine besylate and 300 µg/ml of Indapamide.

b) Preparation of Placebo solution

0.9825 g of placebos were accurately weighed and transferred in to a 100 ml flask and added sufficient volume of methanol to dissolve the substance and made up to the mark with same. From the above solution, 10 ml was transferred in to a 100 ml flask and made up to mark with mobile phase.

c) Procedure

To each 10 mL of 50 % preanalysed placebos solution added, 8, 10 and 12 mL of Amlodipine besylate and Indapamide raw material stock solutions into three different 100 mL volumetric flasks and made up to the mark with mobile phase. The procedure was repeated as per analysis of placebo. The quantity of raw material recovered was calculated by using slope and intercept values from the calibration graph.

Limit of Detection and Limit of Quantification

Preparation of calibration curve for the serial dilution of standard was repeated for six times. The limit of detection and limit of quantification were calculated by using the average value of slope and standard deviation of response (Intercept).

System suitability studies

The system suitability studies conceded as per ICH guidelines and USP. The parameters such as capacity factor, tailing factor, asymmetry factor, number of theoretical plate and resolution were calculated.

RESULTS AND DISCUSSION

5. RESULTS AND DISCUSSION

Estimation of multiple drugs in formulations have advantage over the methods which are time consuming and also usage of solvent is minimized. Two simple, rapid, precise and accurate Spectrophotometric methods and an isocratic RP – HPLC method were developed and validated for the estimation of Amlodipine besylate and Indapamide in pure form and in combined tablet dosage form. The methods employed which are

- UV Spectrophotometric methods

  Derivative Spectrophotometric method

- RP – HPLC method

5.1 DERIVATIVE SPECTROPHOTOMETRIC METHOD

A simple, accurate and precise Derivative spectrophotometric method was developed and validated for the simultaneous estimation of Amlodipine besylate and Indapamide in combined dosage form. The drugs were identified by the melting point and IR spectrum, the IR spectrum of Amlodipine besylate and Indapamide are given in figures 1 and 2, respectively. The solubility of the drugs in various polar and non polar solvents was checked as per the I.P specifications. The common solvent used for estimation of Amlodipine besylate and Indapamide was chosen is methanol followed by 0.2M Borate buffer - pH 8.0. The solubility profile of Amlodipine besylate and Indapamide are given in Tables 1 and 2 respectively.

The sample solutions of 10 µg/ml of Amlodipine besylate and Indapamide in methanol prepared individually and the solutions were scanned in UV region in the wavelength range from 200 and 400 nm by using methanol and 0.2M Borate buffer

pH 8.0 as blank. The zero order spectrums were derivatised into first order derivative spectrum. The first order derivative spectrum of Amlodipine besylate and Indapamide was recorded as shown in Figure 3, 4 and 5 respectively. From the spectrum, 339.0 nm and 293.0 nm were selected for the estimation of Amlodipine besylate and Indapamide respectively without any interference. At 293.0 nm, Amlodipine besylate has zero absorbance. At 339.0 nm, Indapamide has zero absorbance value. Hence these two wavelengths were selected for the analysis of Amlodipine besylate and Indapamide, respectively. Different aliquots of Amlodipine besylate and Indapamide were prepared in the concentration range of 15 - 90 µg/ml and 5- 30 µg/ml, respectively. The absorbances of these solutions were measured at 339.0 nm and 293.0 nm in the first order derivative spectrum for Amlodipine besylate and Indapamide, respectively. The plotted graphs are shown in Figure 6 and 7 for Amlodipine besylate and Indapamide, respectively. The preparation of calibration curve was repeated for six times for each drug at their selective wavelength. The calibration curve was plotted using concentration against absorbance. The optical parameters like, Sandell's sensitivity, Molar absorptivity, correlation coefficient, slope, intercept, LOD, LOQ and Standard error were calculated for the two drugs. The correlation coefficient for the two drugs was found to be above 0.999. This indicates that the two drugs obey Beer's law in the selected concentration range. Hence the concentrations were found to be linear. The results are shown in Table 3.

Formulated tablets containing 5 mg of Amlodipine besylate and 1.5 mg of Indapamide was selected for analysis. The solution contains 25 µg/ml of Amlodipine besylate was prepared (nominal concentration in the calibration curve of Amlodipine besylate), which is also contains 7.5 µg/ml of Indapamide, the absorbance of these solutions were measured at 339.0 nm and 293.0 nm. The amount of six test solutions

was determined. The percentage label claim present in tablet formulation was found to be 98.60 ± 0.6196 and 99.325 ± 1.2554 for Amlodipine besylate and Indapamide, respectively. The amount present in tablet formulation was in good concord with the label claim and the % RSD values were found to be 0.6247 and 1.26398 for Amlodipine besylate and Indapamide, respectively. The results of analysis are shown in Table 4. The low % RSD values indicate that the method has good precision.

Further the precision of the method was confirmed by Intra-day and Inter-day analysis. The analysis of formulation was carried out for three times in the same day and one time in the three consecutive days. The % RSD value of Intraday and Inter day analysis are 0.7026 and 0.1175 for Amlodipine besylate, 1.1703 and 1.1783 for Indapamide, respectively. The results of analysis are shown in Table 5. Hence the precision was confirmed. The results showed that the precision of the method was further confirmed.

The developed method was validated for Ruggedness. It refers to the specific of one lab to multiple days which may include multiple analysts, multiple instruments and different sources of reagents and so on. In the present work it was confirmed by different analysts. The % RSD value by analyst 1 and analyst 2 were found to be 0.1174 and 0.1178 for Amlodipine besylate and 0.1783 and 1.1703 for Indapamide, respectively. The low % RSD values indicate that the developed method was more rugged. The results are shown in Table 6.

The accuracy of the method was performed by recovery studies. To the preanalyzed formulation, a known quantity of Amlodipine besylate and Indapamide raw material solutions were added at different levels. The absorbance of the solutions was measured and the percentage recovery was calculated. The percentage

recovery was found to be in the range of 98.14 - 99.22% for Amlodipine besylate, and 99.24 – 100.56% for Indapamide. The low % RSD value for the two drugs indicates that this method is very accurate. The recovery data is shown in Table 7.

5.2 RP – HPLC METHOD

An exertion has been made for a simple, rapid, accurate and precise method for the estimation of Amlodipine besylate and Indapamide pure form and in formulation by an isocratic RP – HPLC method.

The solutions of 10 µg/ml of Amlodipine besylate and Indapamide in mobile phase Acetonitrile: Methanol: Phosphate buffer - pH 3.0 (25:30:45 % v/v) were prepared and the solutions were scanned in the range of 200 – 400 nm. It was found that the two drugs have marked absorbance at 240 nm and can be effectively used for estimation of two drugs without interference. Therefore 240 nm was selected as detection wavelength for estimation of two drugs by RP – HPLC method with an isocratic elution technique and it was found that the two drugs are stable for three hours. The Spectral confirmation of Amlodipine besylate and Indapamide were shown in Figure 8 and 9 respectively, and the overlain spectrum of Amlodipine besylate and Indapamide in mobile phase is given in Figure 10.

The optimization was done by changing the composition of mobile phase consists of Acetonitrile: Methanol: Phosphate buffer with different ratio was initially attempted and chromatograms were recorded. These are shown in Figure 11 and 12. Finally the mobile phase consists of Acetonitrile: Methanol: 0.04M Phosphate buffer pH 3.0 (25:30:45 v/v/v). After calculating all system suitability parameters Acetonitrile: Methanol: Phosphate buffer pH 3.0 with the ratio of (25:30:45 % v/v/v)

at a flow rate of 1.0 mL/min was selected and the optimized chromatogram is shown in Figure 13. The retention time of Amlodipine besylate and Indapamide, were found to be 8.6 and 6.5, respectively. The retention time between two drugs indicate that the drugs were separated with better resolution of 2.07 Amlodipine besylate and Indapamide. The system suitability parameters for the optimized chromatogram are shown in Table 12.

With the optimized chromatographic conditions, stock solutions of Amlodipine besylate and Indapamide were prepared as the mixture of Amlodipine besylate and Indapamide in the concentration range of 80-120 µg/ml of Amlodipine besylate, 24-36 µg/ml of Indapamide. 20 µL of each solution was injected and recorded the chromatograms at 240 nm. The chromatograms are shown in Figure 14 - 18. The calibration curve was plotted using concentration against peak area. The procedure was repeated for three times. The correlation co-efficient was found to be above 0.999 for the two drugs. The calibration graph of Amlodipine besylate and Indapamide are shown in Figure 19 and 20, respectively. The optical parameters like correlation coefficient, slope, intercept, LOD, LOQ and Standard error were calculated for the two drugs. The above optical parameters were shown in Table 8.

Formulated tablets containing Amlodipine besylate equivalent to 5 mg of Amlodipine and 1.5 mg of Indapamide was selected for analysis. The ostensible concentration 100 µg mL^{-1} of Amlodipine Besylate, which is also contains 30 µg mL^{-1} of Indapamide in the mobile phase was prepared. 20 µL of each solution was injected and chromatograms were recorded. The percentage purity was found to be 100.86 ± 0.7023 and 100.61 ± 0.4471 for Amlodipine besylate and Indapamide, respectively. The precision of the method was confirmed by repeatability of formulation for six times and the chromatograms are shown in Figure 21 – 26. The % RSD was found to

be 0.6963 and 0.4444 for Amlodipine besylate and Indapamide, respectively. It indicates that the method has good precision. The data is shown in Table 9.

The ruggedness of the method was validated by using different analysts and different instruments. The %RSD for analyst 1 and analyst 2 were found to be 0.141322 and 0.748171 for Amlodipine besylate, 0.556417 and 1.230012 for Indapamide, respectively. The results of the analysis are given in Table 11.

The accuracy of the method was performed by recovery studies. To the preanalysed placebos, a known quantity of Amlodipine besylate and Indapamide raw material solutions were added at different levels, injected the solutions. The chromatograms were recorded as shown in the Figure 27–29. The percentage recovery was found to be in the range between 98.71 –100.06 % for Amlodipine besylate and 98.85– 100.36% for Indapamide. The % RSD was found to be 1.783 and 0.650 for Amlodipine besylate and Indapamide, respectively. The low % RSD values for recovery indicated that the method was found to be accurate. The values are given in the Table 10.

All the above parameters with the ease of operation ensure that the proposed methods could be applied for the routine analysis of Amlodipine besylate and Indapamide in pure form and in tablet dosage forms.

SUMMARY & CONCLUSION

6. SUMMARY AND CONCLUSION

Simple, rapid, precise and accurate UV Spectrophotometric method and RP-HPLC method were developed and validated for the estimation of Amlodipine besylate and Indapamide in tablet dosage form.

6.1. UV SPECTROSCOPIC METHODS

From the solubility profile and stability studies, methanol followed by Borate buffer pH 8.0 was chosen as a common solvent for the estimation of Amlodipine besylate and Indapamide. The sample solutions of 10 µg/ml of Amlodipine besylate and Indapamide in methanol followed by Borate buffer - pH 8.0 prepared individually and the solutions were scanned in the wavelength range from 200 to 400 nm by using methanol and 0.2M Borate buffer - pH 8.0 as blank. The overlaid spectra of mixture of Amlodipine besylate and Indapamide were recorded. From the spectra, 339.0 nm for Amlodipine besylate and 293.0 nm for Indapamide were selected as wavelength.

The same spectrums were derivatised and 339.0 nm selected for detection of Amlodipine besylate where Indapamide shows zero crossing and also 293.0 nm selected for detection Indapamide where Amlodipine besylate shows zero crossing. The percentage label claim present in the formulation was found to be 98.60 ± 0.6196 and 99.325 ± 1.2554 for Amlodipine besylate and Indapamide respectively. The percentage recovery was found to be in the range of 98.03- 99.67% for Amlodipine besylate, and 98.30 – 101.10% for Indapamide.

6.2. RP – HPLC METHOD

In RP-HPLC method, mobile phase used is Acetonitrile: Methanol: 0.04M Phosphate buffer - pH 3.0 (25:30:45 v/v/v) with flow rate of 1.0 mL per min, the retention time of Amlodipine besylate and Indapamide were found to be 8.77 and 6.62, respectively at 240.0 nm.

The percentage purity was found to be 100.86 ± 0.7023 and 100.61± 0.4471 for Amlodipine besylate and Indapamide, respectively. The precision of the method was confirmed by repeatability of formulation for six times. The accuracy of the method was confirmed by recovery studies. The percentage recovery was found to be in the range between 100.06 and 101.65 % for Amlodipine besylate and 100.4 and 101.39% for Amlodipine besylate and Indapamide. The low % RSD values for recovery indicated that the method was found to be accurate.

Simple, rapid and accurate UV Spectroscopic (First order derivative method) and an isocratic RP – HPLC methods showed excellent sensitivity, reproducibility, accuracy, and repeatability, which is evidenced by low percentage relative standard deviation. The results obtained in recovery studies were indicating that there is no interference from the excipients used in the formulation. By comparing two methods, UV Spectroscopic methods were found to be economic when compared to RP-HPLC. Hence it is suggested that the proposed UV Spectroscopic and an isocratic RP-HPLC methods can be effectively applied for the routine analysis of Amlodipine besylate and Indapamide in bulk and in tablet formulation. The results drawn are presented as in Figures/Tables at an appropriate place i.e. in the form of sequence viz. identification of samples to recovery Studies including system suitability test parameters.

FIGURES

FIGURE - 1

IR SPECTRUM OF AMLODIPINE BESYLATE

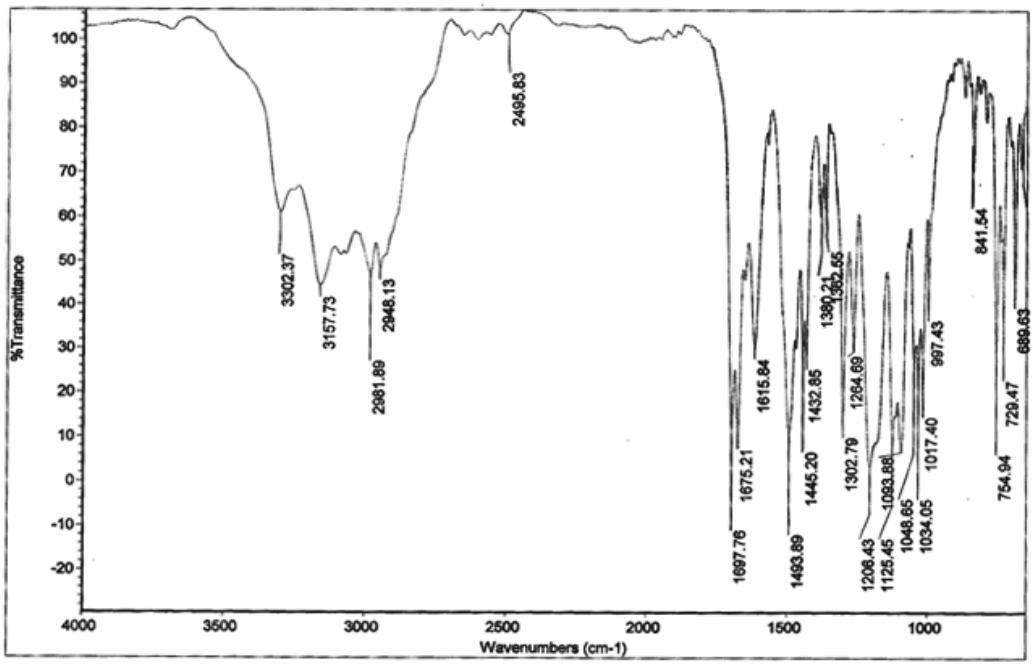


FIGURE - 2

IR SPECTRUM OF INDAPAMIDE

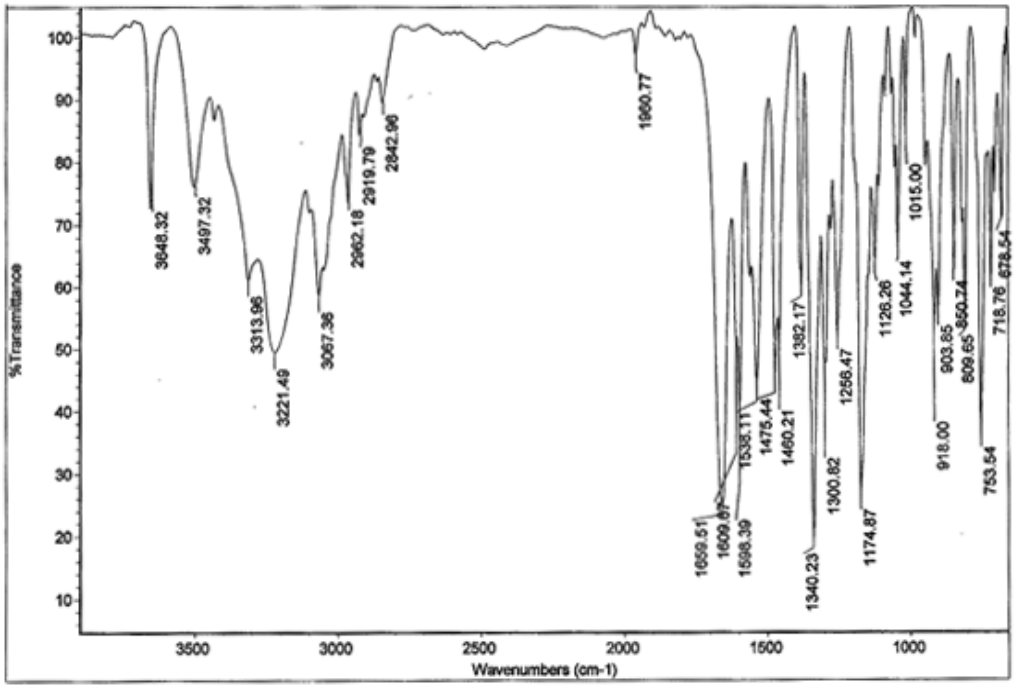


FIGURE - 3

FIRST ORDER DERIVATIVE UV SPECTRUM OFAMLODIPINE BESYLATE IN METHANOL FOLLOWED BY 0.2M BORATE BUFFER - pH 8.0

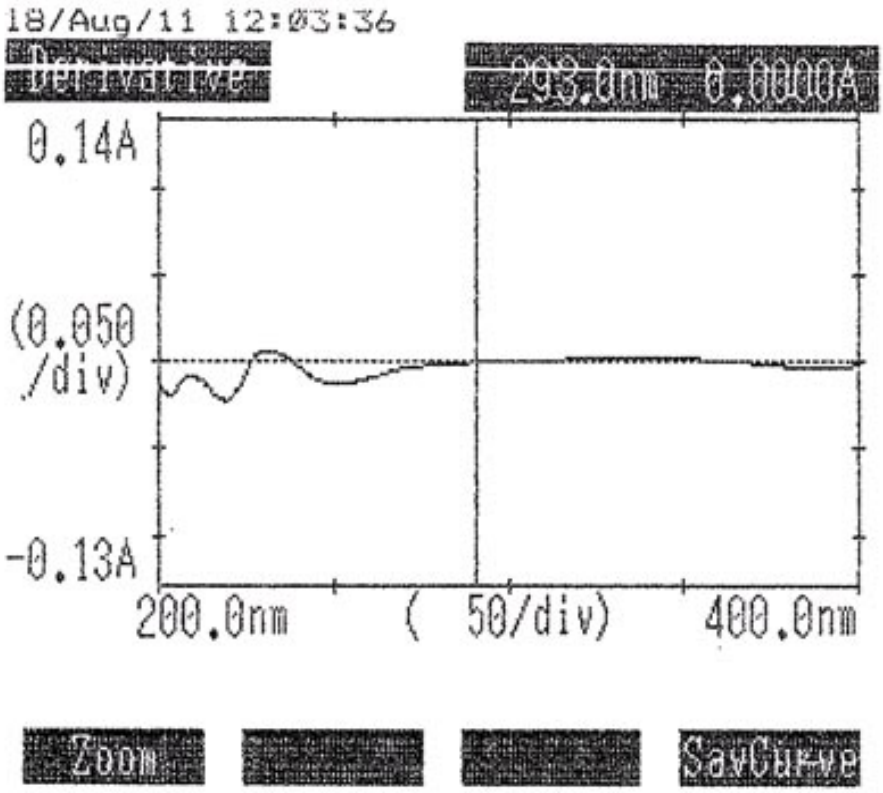


FIGURE - 4

FIRST ORDER DERIVATIVE UV SPECTRUM OF INDAPAMIDE IN METHANOL FOLLOWED BY 0.2M BORATE BUFFER - pH 8.0

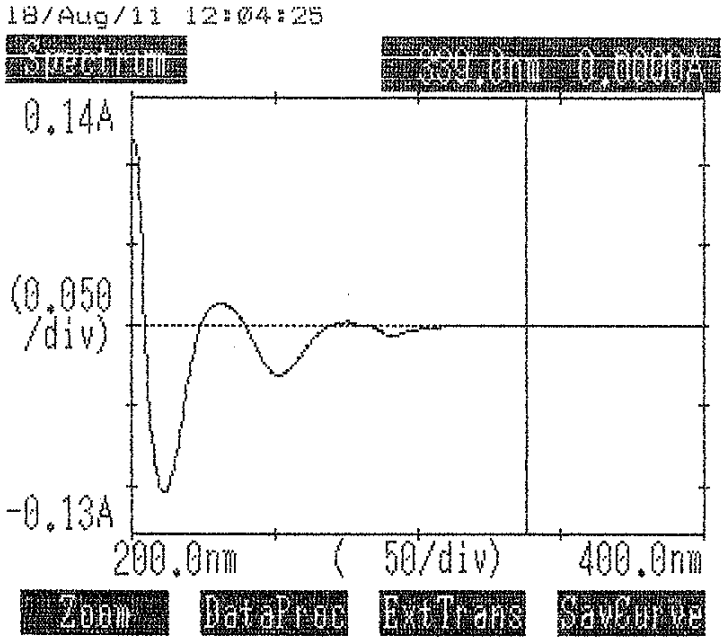


FIGURE – 5

OVERLAIN FIRST ORDER DERIVATIVE UV SPECTRUM OF AMLODIPINE BESYLATE AND INDAPAMIDE IN METHANOL FOLLOWED BY 0.2M BORATE BUFFER - pH 8.0

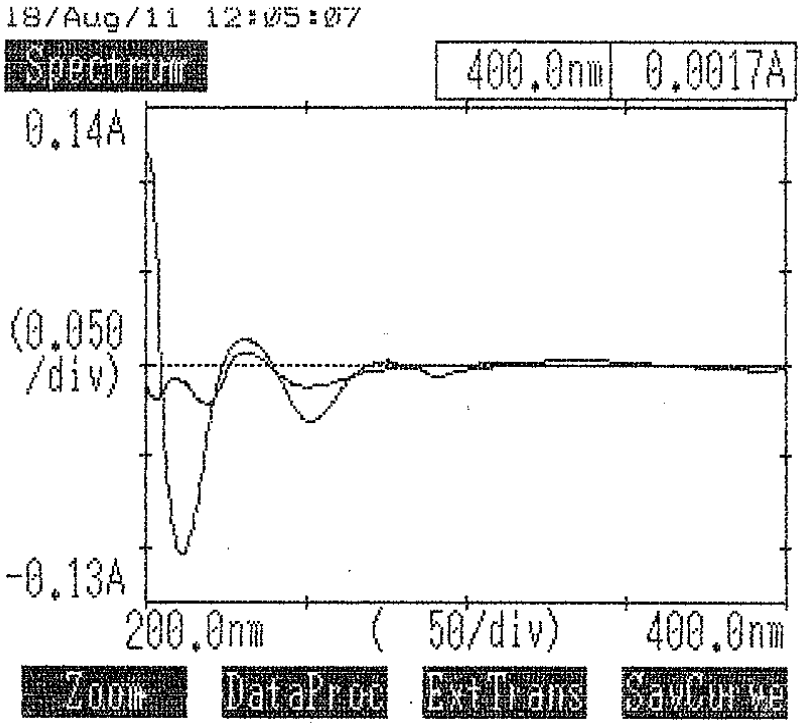


FIGURE - 6

CALIBRATION CURVE OF AMLODIPINE BESYLATE IN METHANOL

FOLLOWED BY BORATE BUFFER AT 339.0

(FIRST ORDER DERIVATIVE SPECTROPHOTOMETRIC METHOD)

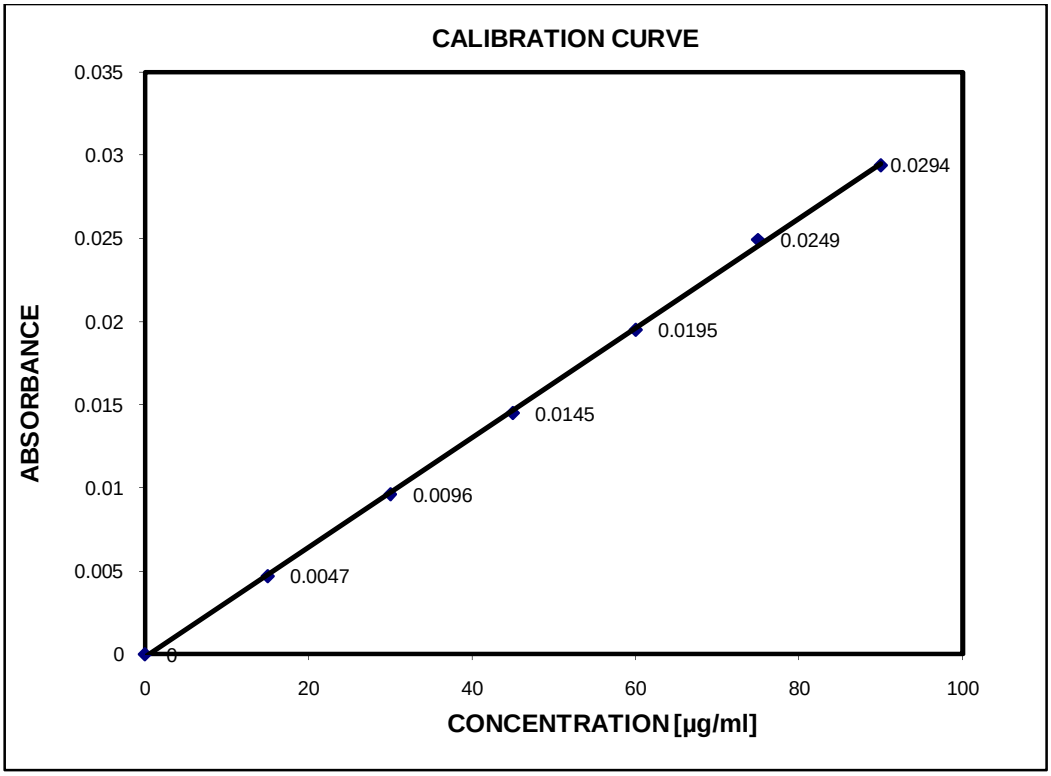


FIGURE-7

CALIBRATION CURVE OF INDAPAMIDE IN METHANOL FOLLOWED BY BORATE BUFFER AT 293.0 (FIRST ORDER DERIVATIVE SPECTROPHOTOMETRIC METHOD)

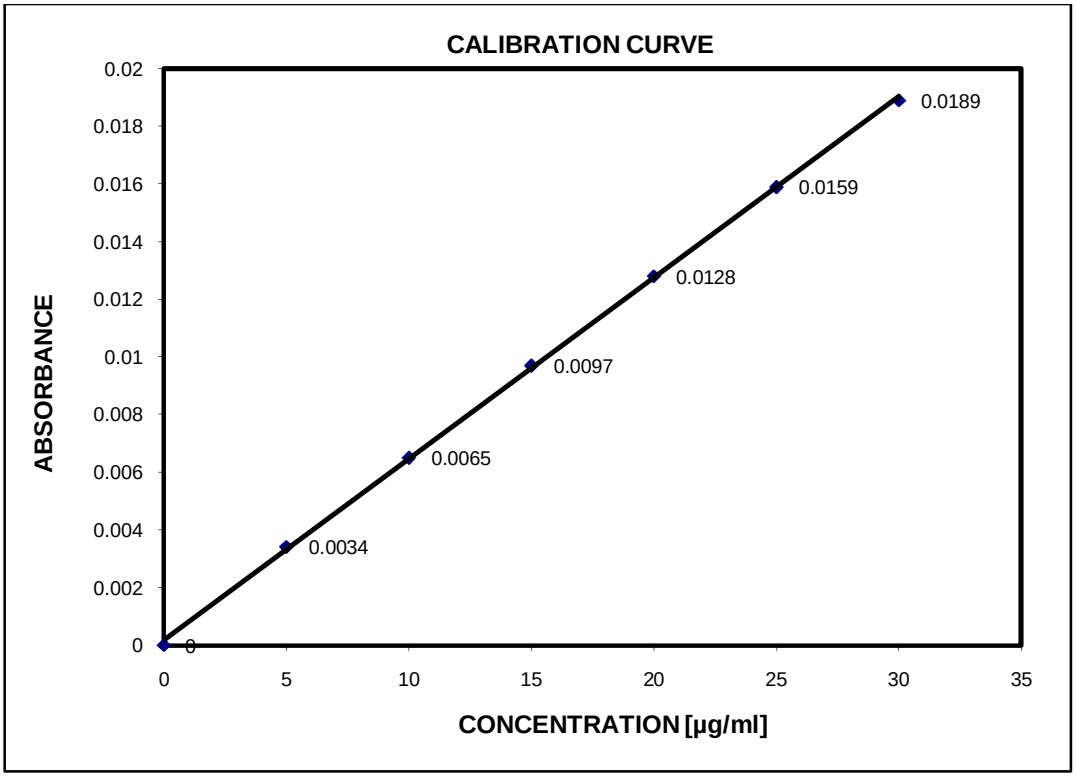
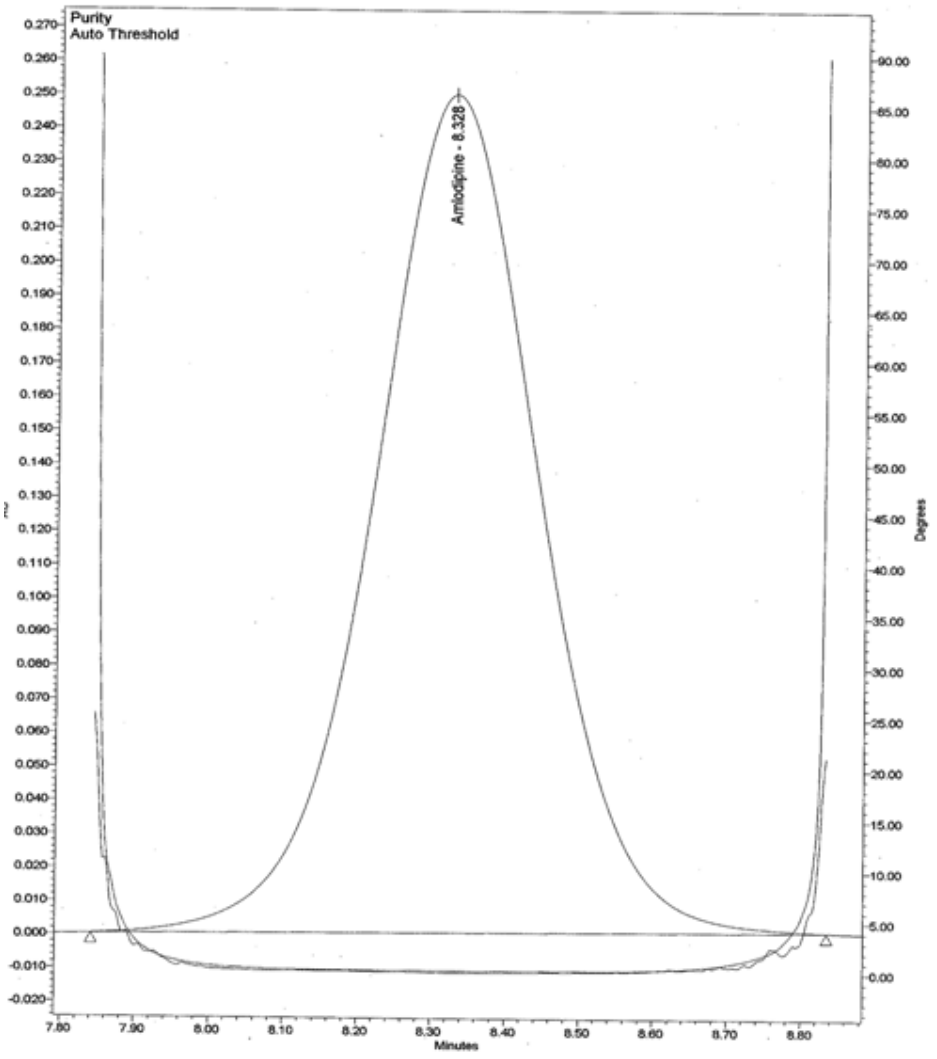


FIGURE - 8

SPECTRAL CONFIRMATION OF AMLODIPINE BESYLATE IN METHANOL FOLLOWED BY MOBILE PHASE AT 240.0 nm

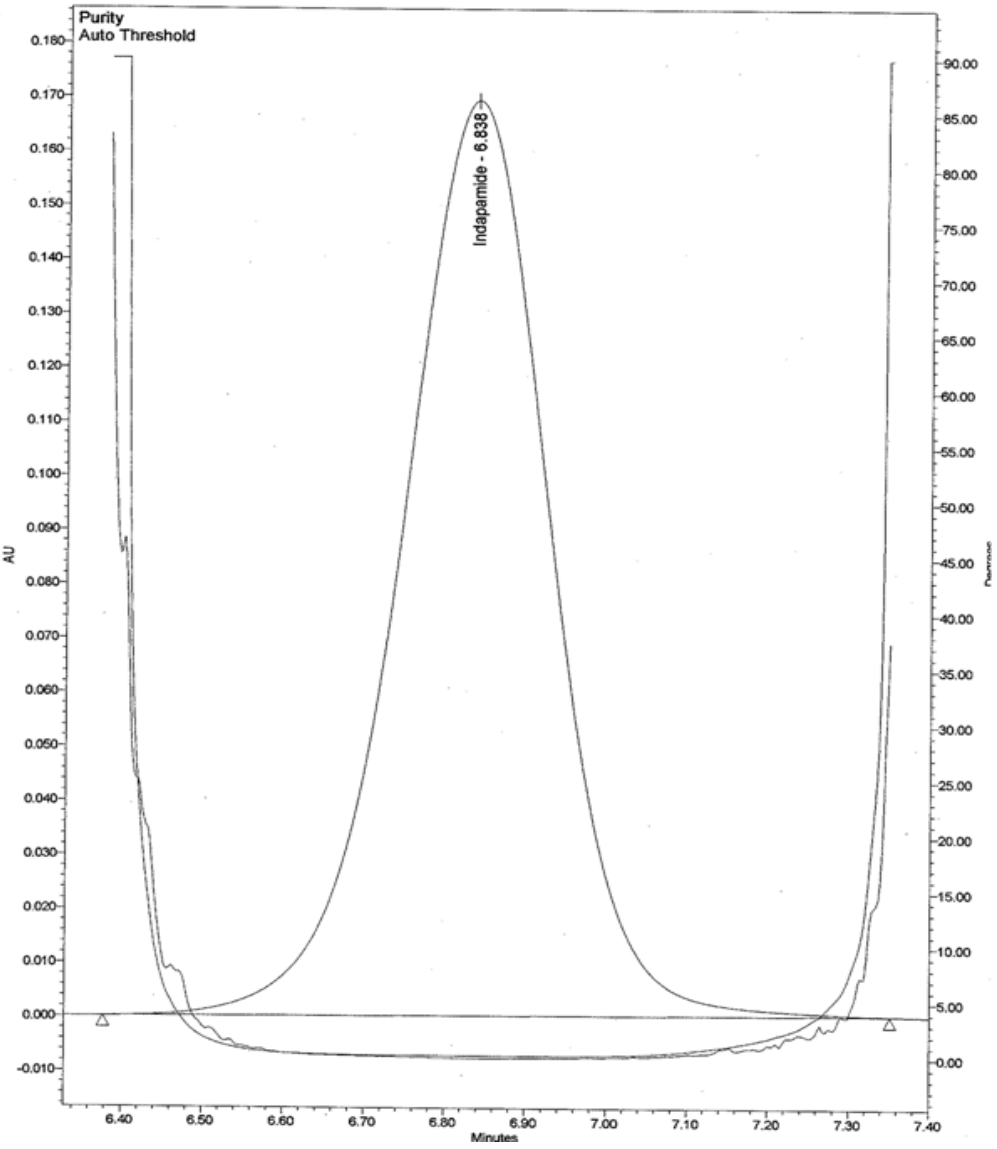


	Purity Angle	Purity Threshold	Maximum Impurity	Purity Flag
1	0.066	0.219	8.236	

FIGURE - 9

SPECTRAL CONFIRMATION OF INDAPAMIDE IN METHANOL

FOLLOWED MOBILE PHASE AT 240.0 nm



	Purity Angle	Purity Threshold	Maximum Impurity	Purity Flag
1	0.068	0.240	6.738	

FIGURE -10

OVERLAIN SPECTRUM OF AMLODIPINE BESYLATE AND INDAPAMIDE IN METHANOL AND MOBILE PHASE AT 240.0 nm

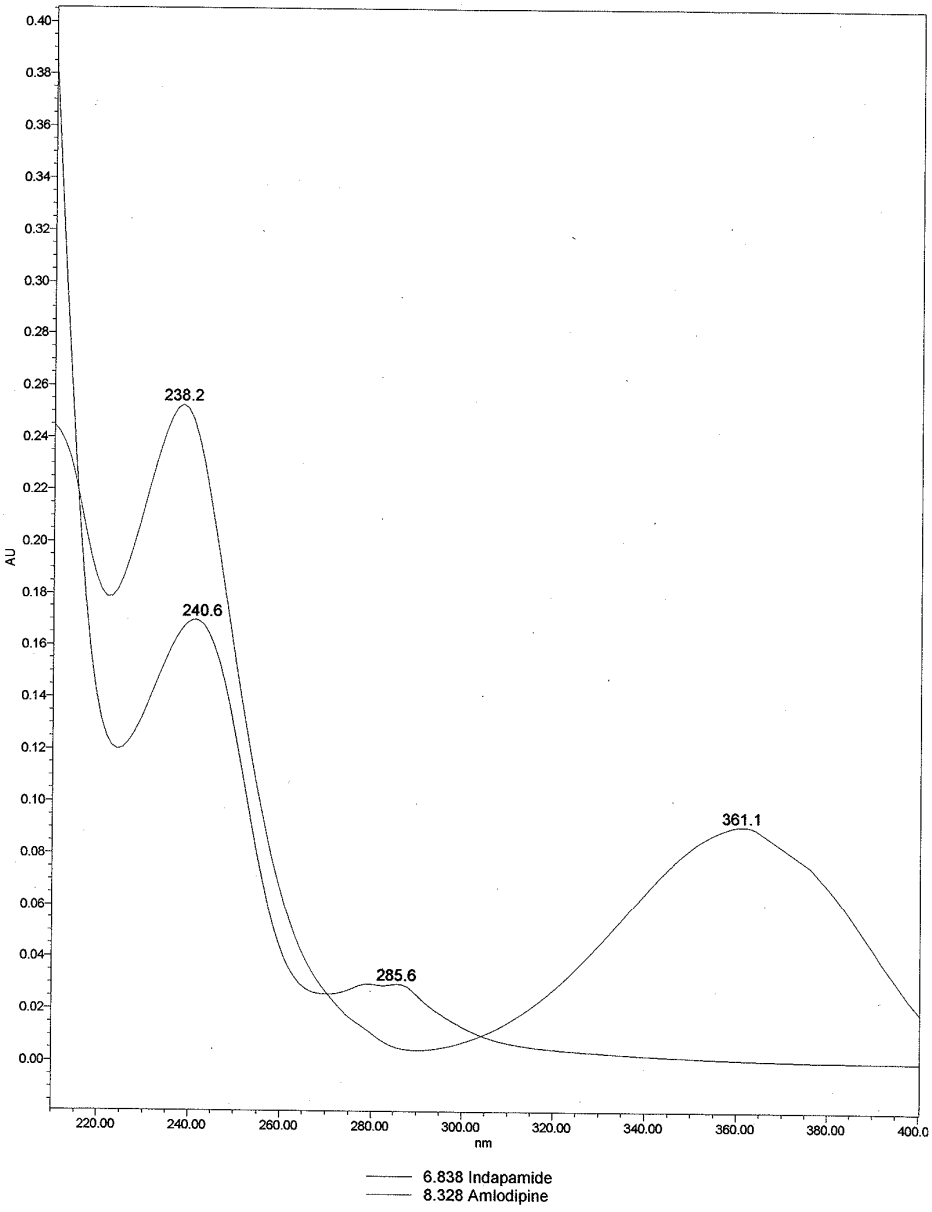


FIGURE -11

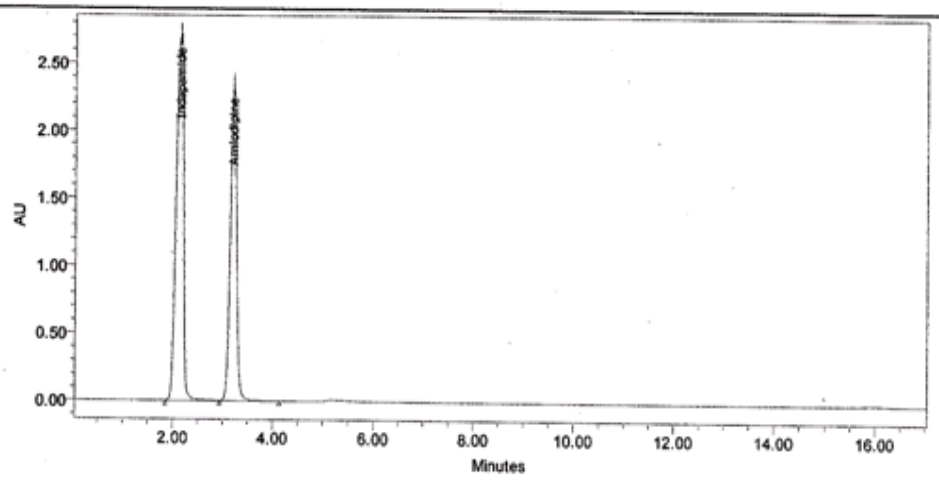
CHROMATOGRAM OF AMLODIPINE BESYLATE AND INDAPAMIDE IN METHANOL AND ACETONITRILE (50: 50 % v/v)

Empower 2 Software

Component Summary Report

SAMPLE INFORMATION

Sample Name:	INDAP+AMLO STD1 W&M	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	
Vial:	2	Acq. Method Set:	Isocratic PDA _MS
Injection Volume:	20.00 ul	Run Time:	17.0 Minutes



Name: Amlodipine

	Name	RT	Area
1	Amlodipine	3.201	20462834
Mean		3.201	20462834.313
Std. Dev.			
% RSD			

Name: Indapamide

	Name	RT	Area
1	Indapamide	2.138	28054818
Mean		2.138	28054818.434
Std. Dev.			
% RSD			

FIGURE-12

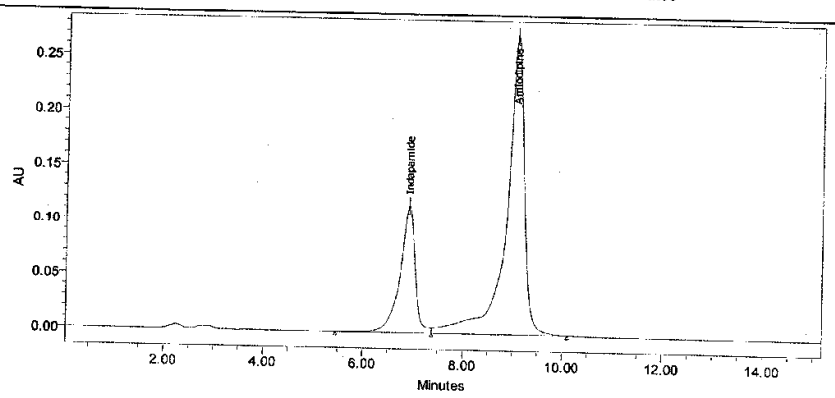
CHROMATOGRAM OF AMLODIPINE BESYLATE AND INDAPAMIE IN ACETONITRILE, METHANOL AND PHOSPHATE BUFFER - pH 3.0 (30:40:30 % v/v/v)

Empower2

Component Summary Report

SAMPLE INFORMATION

Sample Name:	INDAP+AMLO STD -with dilmethanol	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	
Vial:	7	Acq. Method Set:	Isocratic PDA _MS
Injection Volume:	20.00 ul	Run Time:	20.0 Minutes



Name: Amlodipine

	Name	RT	Area
1	Amlodipine	9.055	6795147
Mean		9.055	6795147.022
Std. Dev.			
% RSD			

Name: Indapamide

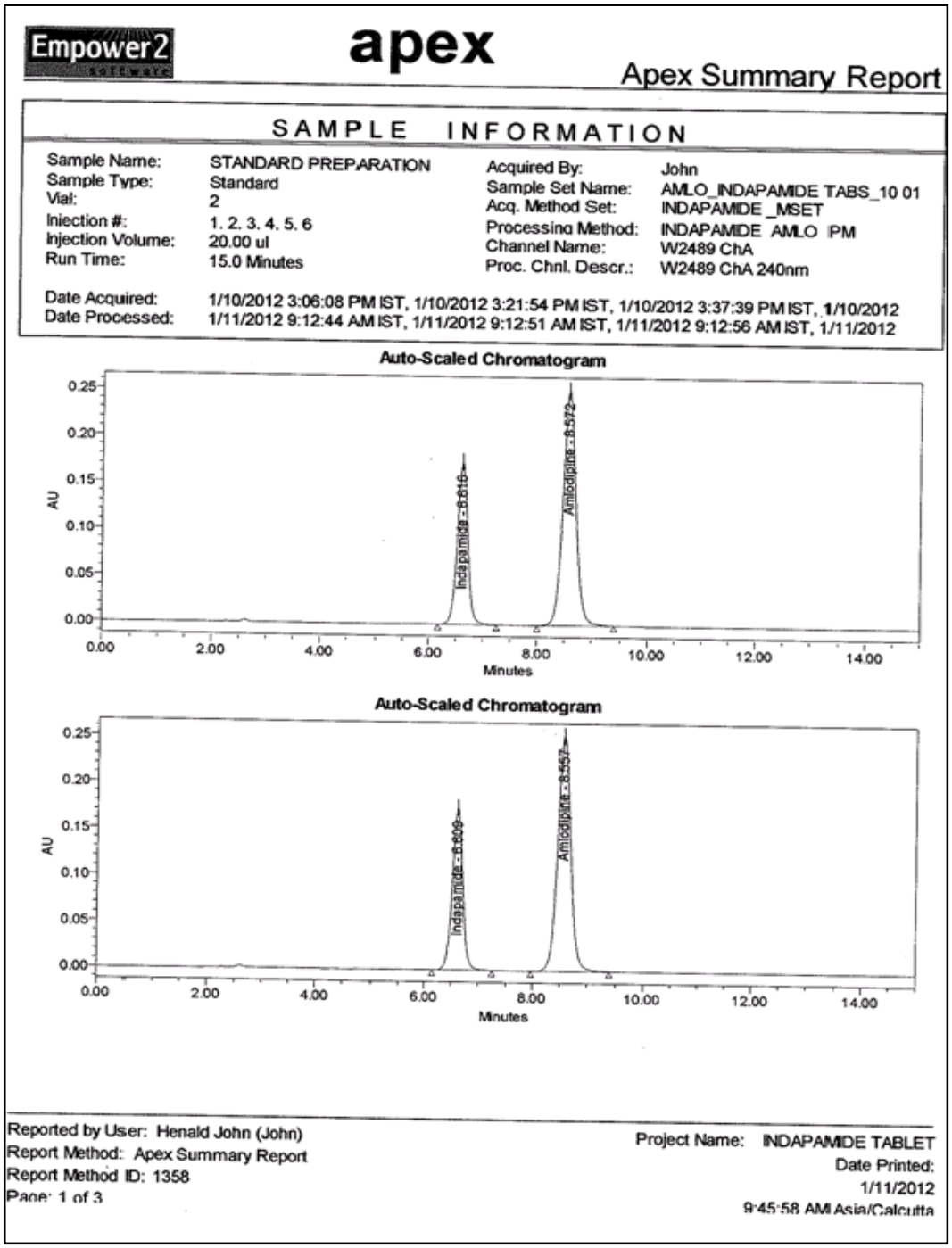
	Name	RT	Area
1	Indapamide	6.918	2429681
Mean		6.918	2429680.571
Std. Dev.			
% RSD			

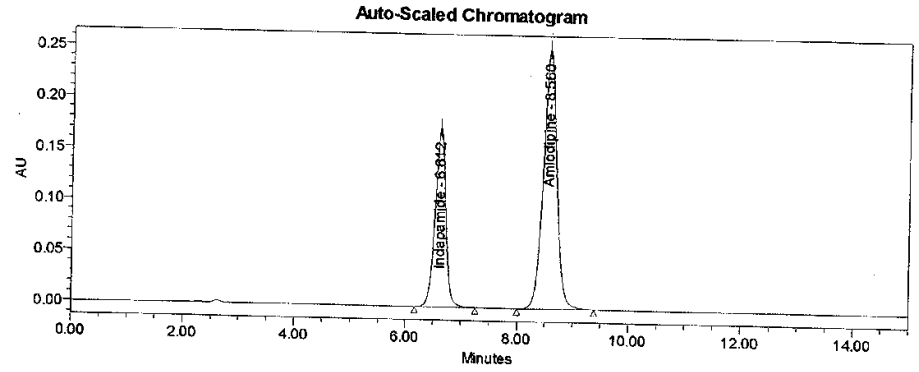
Report Method: Component Summary Printed 1:19:03 PM Asia/Calcutta 12/01/2012 Page: 1 of 1

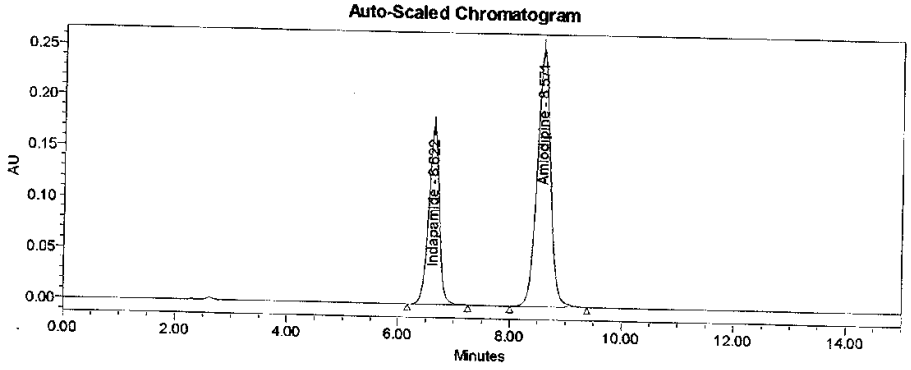
Analysed_by Checked_by

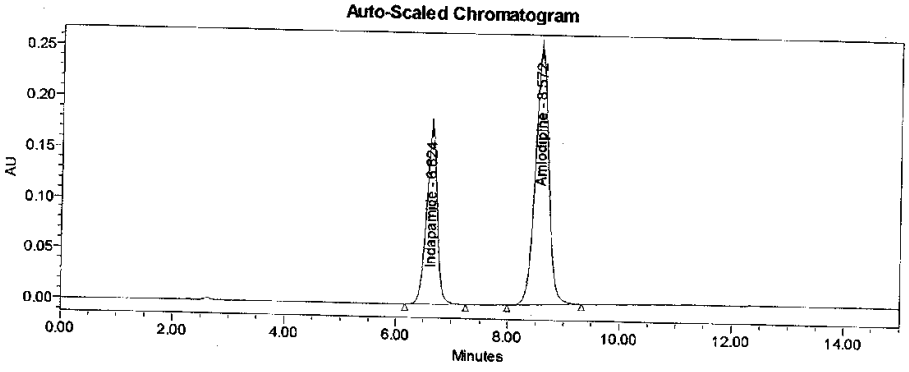
FIGURE-13

OPTIMIZED CHROMATOGRAM OF AMLODIPINE BESYLATE AND INDAPAMIDE IN ACETONITRILE, METHANOL AND PHOSPHATE BUFFER-pH 3.0 (30:35:45 % v/v/v)

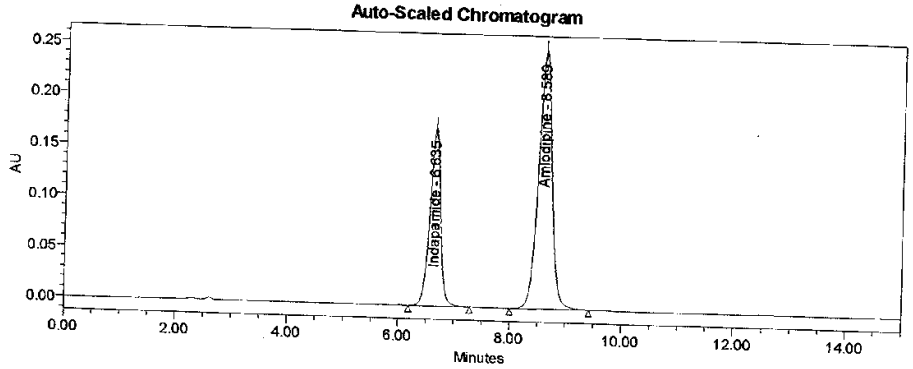



Auto-Scaled Chromatogram
AU
Minutes
Indapamide - 6.812
Amlodipine - 8.560


Auto-Scaled Chromatogram
AU
Minutes
Indapamide - 6.822
Amlodipine - 8.574


Auto-Scaled Chromatogram
AU
Minutes
Indapamide - 6.824
Amlodipine - 8.572

Auto-Scaled Chromatogram



Component Summary Table
Name: Amlodipine

	Sample Name	Name	RT	Area	USP Tailing	USP Plate Count	USP Resolution
1	STANDARD PREPARATION	Amlodipine	8.572	4196387	1.07	6358.85	4.95
2	STANDARD PREPARATION	Amlodipine	8.557	4205839	1.06	6372.02	4.95
3	STANDARD PREPARATION	Amlodipine	8.560	4206083	1.07	6347.52	4.94
4	STANDARD PREPARATION	Amlodipine	8.571	4210750	1.07	6361.40	4.94
5	STANDARD PREPARATION	Amlodipine	8.572	4204146	1.05	6298.31	4.92
6	STANDARD PREPARATION	Amlodipine	8.589	4220845	1.07	6323.19	4.93
Mean			8.570	4207341.490	1.1	6343.5	4.9
Std. Dev.			0.011	8099.553			
% RSD			0.1	0.2			

Component Summary Table
Name: Indapamide

	Sample Name	Name	RT	Area	USP Tailing	USP Plate Count	USP Resolution
1	STANDARD PREPARATION	Indapamide	6.616	2307985	1.00	5930.28	
2	STANDARD PREPARATION	Indapamide	6.609	2312320	0.99	6028.92	
3	STANDARD PREPARATION	Indapamide	6.612	2312990	1.00	5971.71	
4	STANDARD PREPARATION	Indapamide	6.622	2315749	0.99	6011.59	
5	STANDARD PREPARATION	Indapamide	6.624	2315321	0.99	5966.63	
6	STANDARD PREPARATION	Indapamide	6.635	2322285	1.00	5948.18	
Mean			6.620	2314441.492	1.0	5976.2	
Std. Dev.			0.009	4739.294			
% RSD			0.1	0.2			

FIGURE-14

LINEARITY CHROMATOGRAM OF AMLODIPINE BESYLATE AND INDAPAMIDE (80, 24 µg/ml)

Empower 2

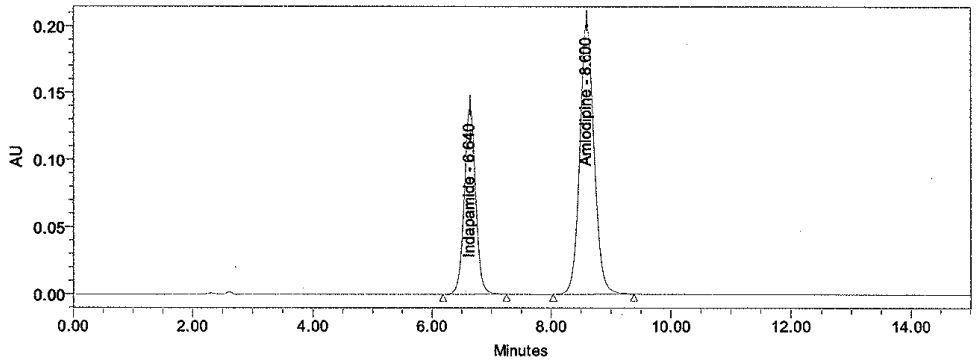
apex

Apex Summary Report

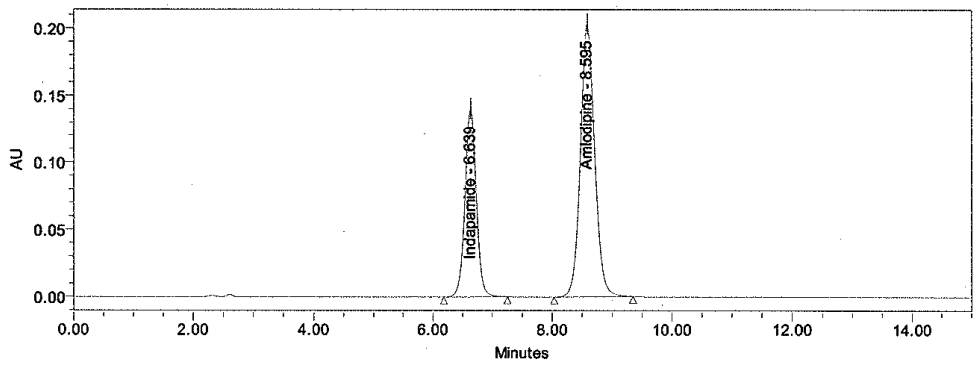
SAMPLE INFORMATION

Sample Name:	LINEARITY 80%	Acquired By:	John
Sample Type:	Standard	Sample Set Name:	AMLO INDAPAMIDE TABS 10 01 12
Vial:	3	Acq. Method Set:	INDAPAMIDE_MSET
Injection #:	1, 2	Processing Method:	INDAPAMIDE AMLO PM
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	15.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 240nm
Date Acquired:	1/10/2012 4:40:43 PM IST, 1/10/2012 4:56:28 PM IST		
Date Processed:	1/11/2012 9:13:44 AM IST, 1/11/2012 9:13:50 AM IST		

Auto-Scaled Chromatogram



Auto-Scaled Chromatogram



Reported by User: Henald John (John)
Report Method: Apex Summary Report
Report Method ID: 1660
Page: 1 of 2

Project Name: INDAPAMIDE TABLET
Date Printed:
1/11/2012
11:03:02 AM Asia/Calcutta

Component Summary Table
Name: Amlodipine

	Sample Name	Name	RT	Area	USP Plate Count	USP Tailing	USP Resolution
1	LINEARITY 80%	Amlodipine	8.600	2765417	6370.55	1.05	4.95
2	LINEARITY 80%	Amlodipine	8.595	2762471	6396.14	1.05	4.95
Mean			8.597	2763944.141	6383.3	1.1	4.9
Std. Dev.			0.004	15835.979			
% RSD			0.0	0.0			

Component Summary Table
Name: Indapamide

	Sample Name	Name	RT	Area	USP Plate Count	USP Tailing	USP Resolution
1	LINEARITY 80%	Indapamide	6.640	1685241	5980.81	1.00	
2	LINEARITY 80%	Indapamide	6.639	1674152	6006.21	1.00	
Mean			6.640	1679696.550	5993.5	1.0	
Std. Dev.			0.001	5729.912			
% RSD			0.0	0.4			

FIGURE-15

LINEARITY CHROMATOGRAM OF AMLODIPINE BESYLATE AND INDAPAMIDE (90, 27 µg/ml)

Empower 2

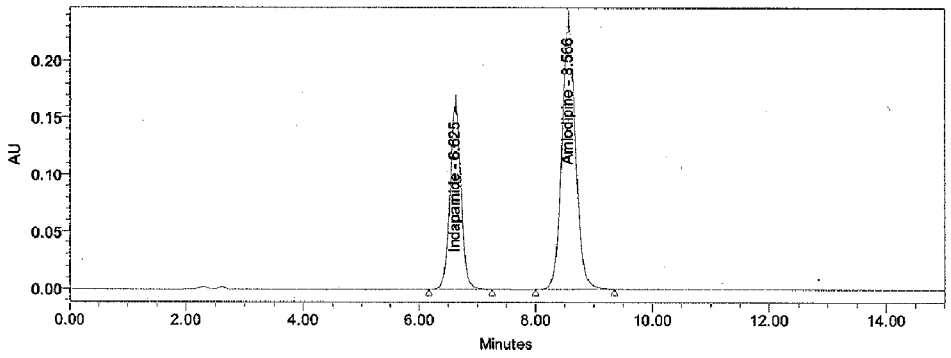
apex

Apex Summary Report

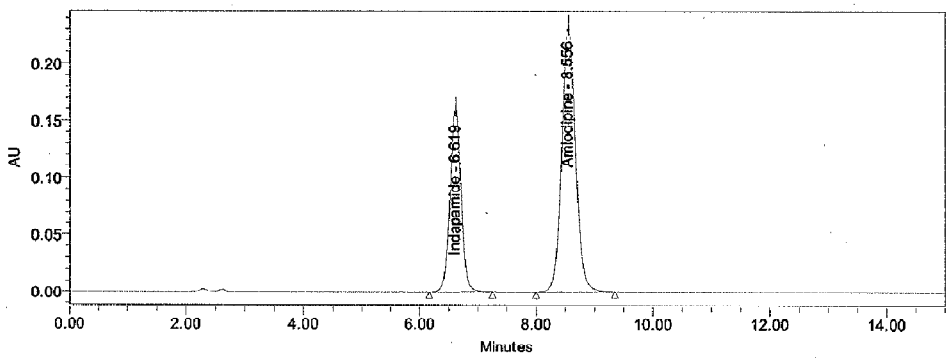
SAMPLE INFORMATION

Sample Name:	LINERITY 90%	Acquired By:	John
Sample Type:	Standard	Sample Set Name:	AMLO INDAPAMIDE TABS 10 01 12
Vial:	4	Acq. Method Set:	INDAPAMIDE_MSET
Injection #:	1, 2	Processing Method:	INDAPAMIDE AMLO PM
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	15.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 240nm
Date Acquired:	1/10/2012 5:12:16 PM IST, 1/10/2012 5:28:03 PM IST		
Date Processed:	1/11/2012 9:13:55 AM IST, 1/11/2012 9:14:00 AM IST		

Auto-Scaled Chromatogram



Auto-Scaled Chromatogram



Component Summary Table
Name: Amlodipine

	Sample Name	Name	RT	Area	USP Plate Count	USP Tailing	USP Resolution
1	LINEARITY 90%	Amlodipine	8.566	3105412	6356.68	1.06	4.92
2	LINEARITY 90%	Amlodipine	8.556	3105741	6325.78	1.06	4.90
Mean			8.561	3105576.501	6341.2	1.1	4.9
Std. Dev.			0.007	1355.019			
% RSD			0.1	0.0			

Component Summary Table
Name: Indapamide

	Sample Name	Name	RT	Area	USP Plate Count	USP Tailing	USP Resolution
1	LINEARITY 90%	Indapamide	6.625	1896574	6015.56	0.99	
2	LINEARITY 90%	Indapamide	6.619	1893654	5935.73	1.00	
Mean			6.622	1895114.001	5975.6	1.0	
Std. Dev.			0.004	124.135			
% RSD			0.1	0.0			

FIGURE-16

LINEARITY CHROMATOGRAM OF AMLODIPINE BESYLATE AND INDAPAMIDE (100, 30 µg/ml)

Empower 2 Software

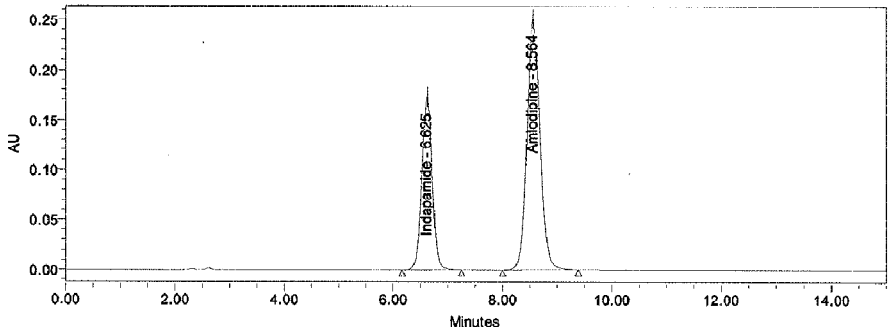
apex

Apex Summary Report

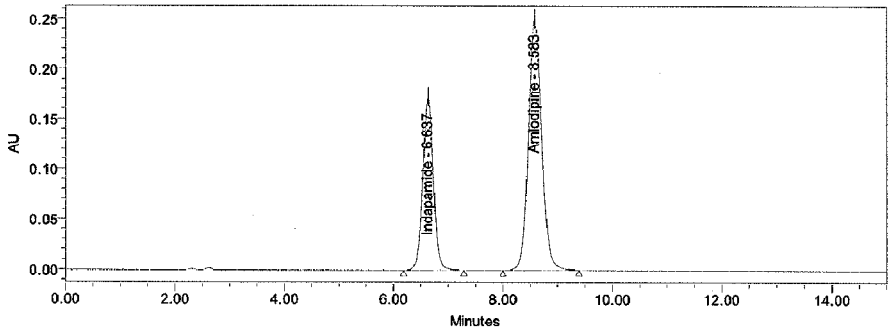
SAMPLE INFORMATION

Sample Name:	LINEARITY 100%	Acquired By:	John
Sample Type:	Standard	Sample Set Name:	AMLO INDAPAMIDE TABS 10 01 12
Vial:	5	Acq. Method Set:	INDAPAMIDE_MSET
Injection #:	1, 2	Processing Method:	INDAPAMIDE AMLO PM
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	15.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 240nm
Date Acquired:	1/10/2012 5:43:49 PM IST, 1/10/2012 5:59:32 PM IST		
Date Processed:	1/11/2012 9:14:04 AM IST, 1/11/2012 9:14:10 AM IST		

Auto-Scaled Chromatogram



Auto-Scaled Chromatogram



Reported by User: Henald John (John)
Report Method: Apex Summary Report
Report Method ID: 1360
Page: 1 of 2

Project Name: INDAPAMIDE TABLET
Date Printed:
1/11/2012
11:10:07 AM Asia/Calcutta

Component Summary Table
Name: Amlodipine

	Sample Name	Name	RT	Area	USP Plate Count	USP Tailing	USP Resolution
1	LINEARITY 100%	Amlodipine	8.564	3453527	6290.54	1.06	4.89
2	LINEARITY 100%	Amlodipine	8.583	3456981	6260.71	1.06	4.88
Mean			8.574	3455254.011	6275.6	1.1	4.9
Std. Dev.			0.013	703.571			
% RSD			0.2	0.1			

Component Summary Table
Name: Indapamide

	Sample Name	Name	RT	Area	USP Plate Count	USP Tailing	USP Resolution
1	LINEARITY 100%	Indapamide	6.625	2125415	5974.65	0.99	
2	LINEARITY 100%	Indapamide	6.637	2123954	5876.73	1.00	
Mean			6.631	2124684.012	5925.7	1.0	
Std. Dev.			0.008	1131.371			
% RSD			0.1	0.1			

FIGURE-17

LINEARITY CHROMATOGRAM OF AMLODIPINE BESYLATE AND INDAPAMIDE (110, 33 µg/ml)

Empower 2

apex

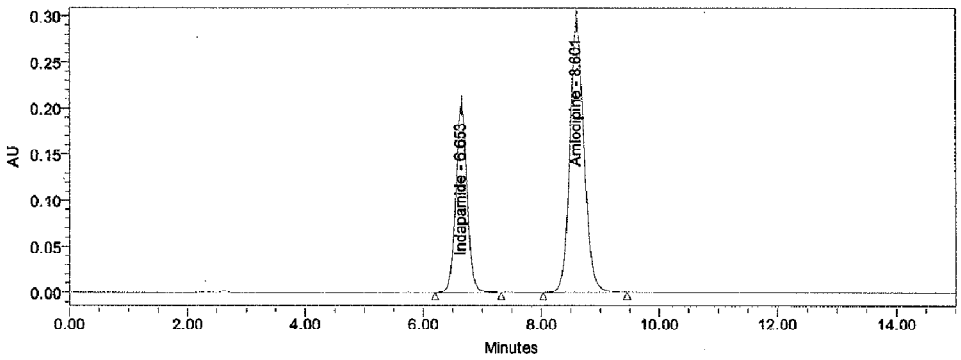
Apex Summary Report

SAMPLE INFORMATION

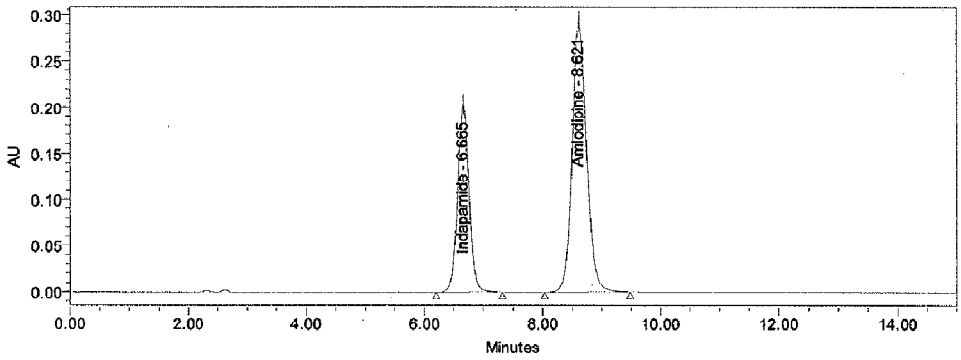
Sample Name:	LINEARITY 110%	Acquired By:	John
Sample Type:	Standard	Sample Set Name:	AMLO INDAPAMIDE TABS 10 01 12
Vial:	6	Acq. Method Set:	INDAPAMIDE_MSET
Injection #:	1, 2	Processing Method:	INDAPAMIDE AMLO PM
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	15.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 240nm

Date Acquired: 1/10/2012 6:15:19 PM IST, 1/10/2012 6:31:06 PM IST
Date Processed: 1/11/2012 9:14:15 AM IST, 1/11/2012 9:14:20 AM IST

Auto-Scaled Chromatogram



Auto-Scaled Chromatogram



Reported by User: Henald John (John)
Report Method: Apex Summary Report
Report Method ID: 1360

Project Name: INDAPAMIDE TABLET
Date Printed:
1/11/2012
11:10:39 AM Asia/Calcutta

Component Summary Table
Name: Amlodipine

	Sample Name	Name	RT	Area	USP Plate Count	USP Tailing	USP Resolution
1	LINEARITY 110%	Amlodipine	8.601	3792687	6201.27	1.08	4.86
2	LINEARITY 110%	Amlodipine	8.621	3792546	6188.90	1.08	4.87
Mean			8.611	3792616.501	6195.1	1.1	4.9
Std. Dev.			0.014	1131.372			
% RSD			0.2	0.0			

Component Summary Table
Name: Indapamide

	Sample Name	Name	RT	Area	USP Plate Count	USP Tailing	USP Resolution
1	LINEARITY 110%	Indapamide	6.653	2334152	5889.43	0.99	
2	LINEARITY 110%	Indapamide	6.665	2325745	5856.47	1.00	
Mean			6.659	2329948.501	5873.0	1.0	
Std. Dev.			0.008	2147.219			
% RSD			0.1	0.1			

FIGURE-18

LINEARITY CHROMATOGRAM OF AMLODIPINE BESYLATE AND INDAPAMIDE (120, 36 µg/ml)

Empower 2

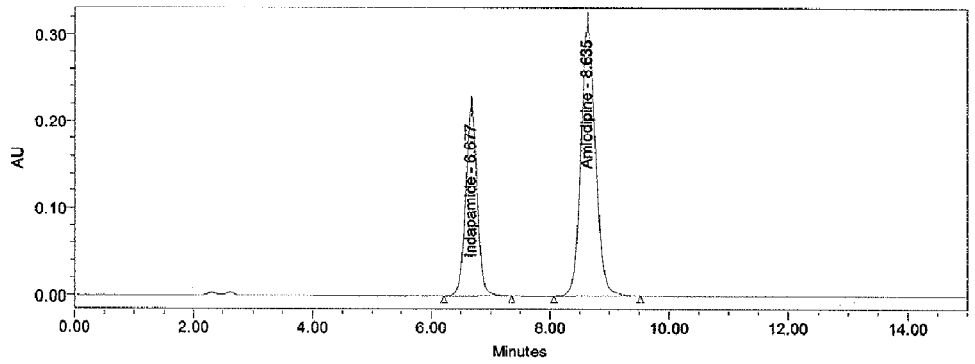
apex

Apex Summary Report

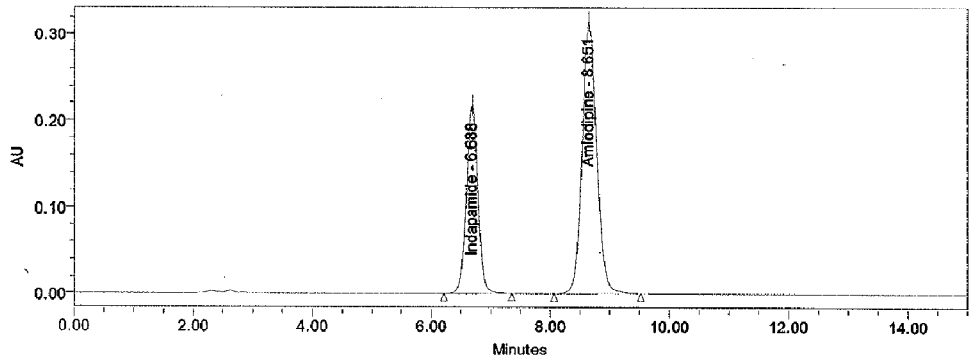
SAMPLE INFORMATION

Sample Name:	LINEARITY 120%	Acquired By:	John
Sample Type:	Standard	Sample Set Name:	AMLO INDAPAMIDE TABS 10 01 12
Vial:	7	Acq. Method Set:	INDAPAMIDE_MSET
Injection #:	1, 2	Processing Method:	INDAPAMIDE AMLO PM
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	15.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 240nm
Date Acquired:	1/10/2012 6:46:54 PM IST, 1/10/2012 7:02:39 PM IST		
Date Processed:	1/11/2012 9:14:25 AM IST, 1/11/2012 9:14:30 AM IST		

Auto-Scaled Chromatogram



Auto-Scaled Chromatogram



Reported by User: Henald John (John)
Report Method: Apex Summary Report
Report Method ID: 1360
Page: 1 of 2

Project Name: INDAPAMIDE TABLET
Date Printed:
1/11/2012
11:10:57 AM Asia/Calcutta

Component Summary Table
Name: Amlodipine

	Sample Name	Name	RT	Area	USP Plate Count	USP Tailing	USP Resolution
1	LINEARITY 120%	Amlodipine	8.635	4143854	6136.96	1.09	4.85
2	LINEARITY 120%	Amlodipine	8.651	4149824	6135.26	1.09	4.85
Mean			8.643	4146839.001	6136.1	1.1	4.9
Std. Dev.			0.011	13964.024			
% RSD			0.1	0.1			

Component Summary Table
Name: Indapamide

	Sample Name	Name	RT	Area	USP Plate Count	USP Tailing	USP Resolution
1	LINEARITY 120%	Indapamide	6.677	2532541	5875.09	1.00	
2	LINEARITY 120%	Indapamide	6.688	2536841	5866.79	1.00	
Mean			6.683	2534691.204	5870.9	1.0	
Std. Dev.			0.007	982.781			
% RSD			0.1	0.0			

FIGURE-19

CALIBRATION CURVE OF AMLODIPINE BESYLATE IN MOBILE PHASE

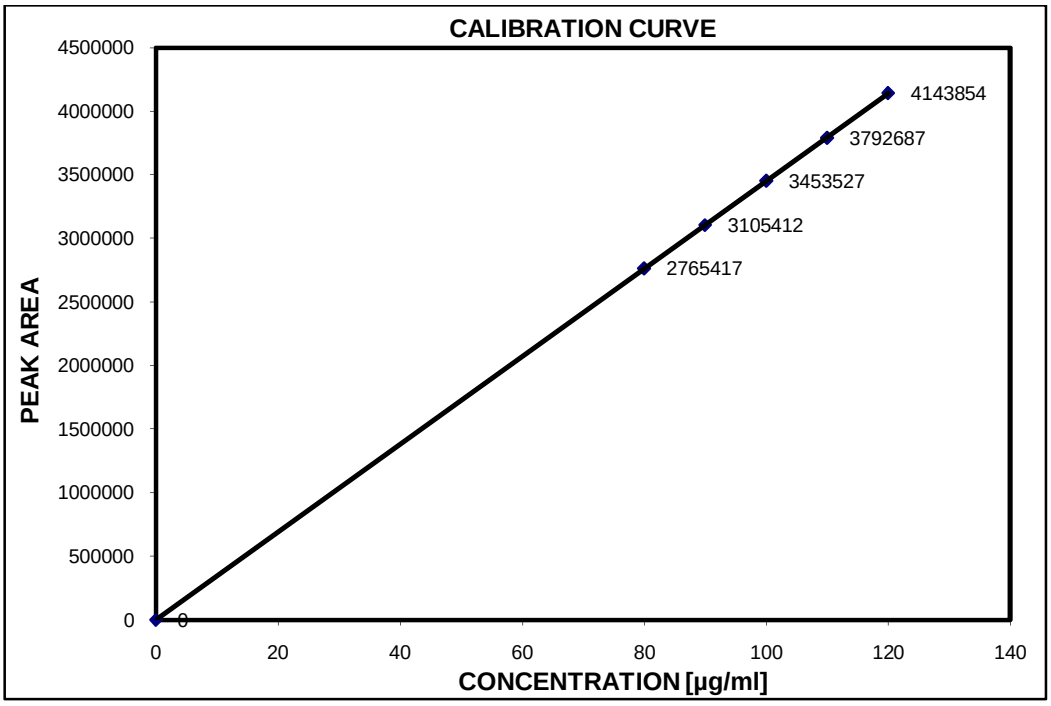


FIGURE-20

CALIBRATION CURVE OF INDAPAMIDE IN MOBILE PHASE

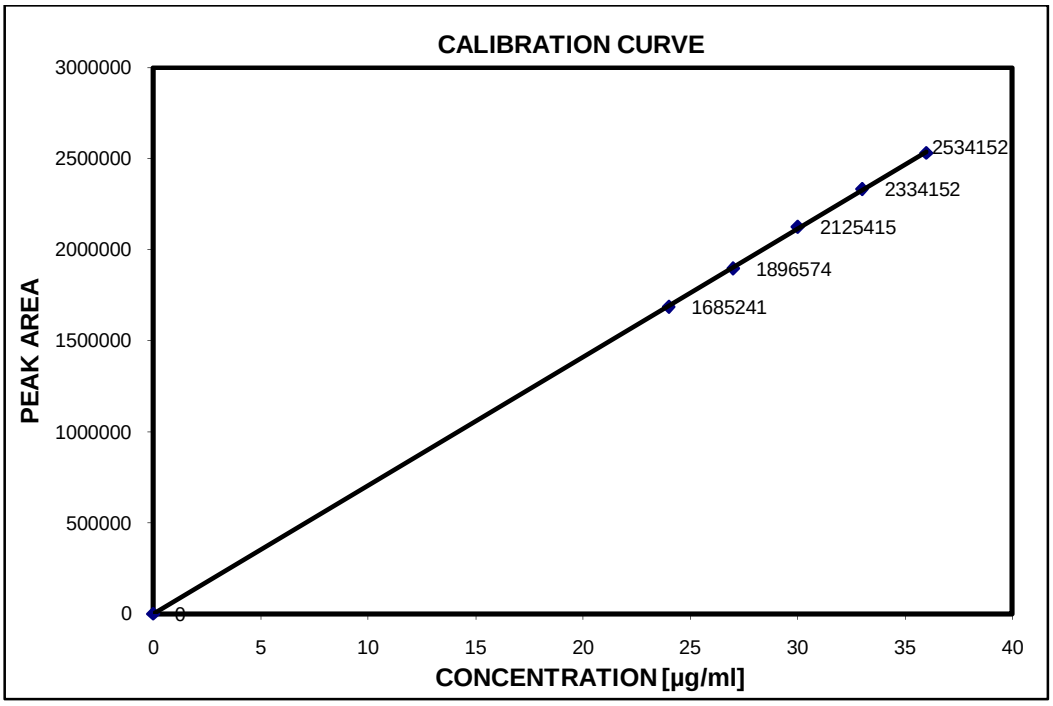


FIGURE-21

CHROMATOGRAM FOR ANALYSIS OF FORMULATION (NATRILAM)

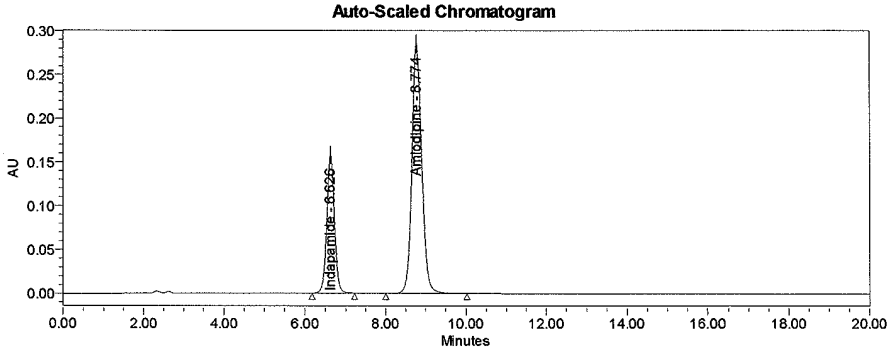
REPEATABILITY - 1

Empower2 **apex** Apex Summary Report

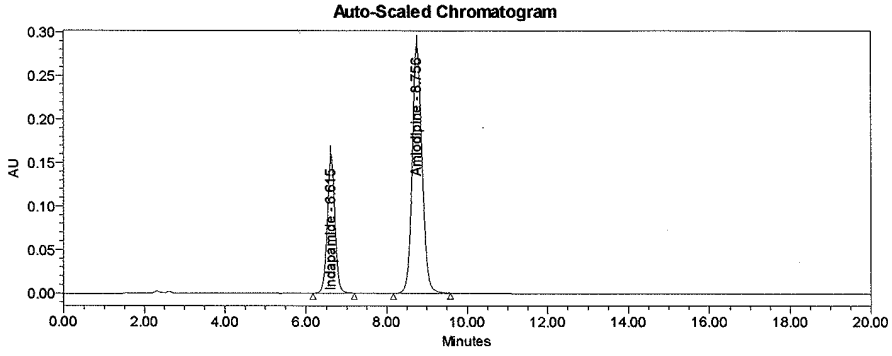
SAMPLE INFORMATION

Sample Name:	Assay preparation -1	Acquired By:	John
Sample Type:	Standard	Sample Set Name:	AMLO_INDAPAMIDE TABS
Vial:	5	Acq. Method Set:	INDAPAMIDE _MSET
Injection #:	1, 2	Processing Method:	INDAPAMIDE AMLO PM
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	20.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 240nm
Date Acquired:	1/9/2012 7:04:35 PM IST, 1/9/2012 7:25:21 PM IST		
Date Processed:	1/10/2012 2:51:26 PM IST, 1/10/2012 2:51:40 PM IST		

Auto-Scaled Chromatogram



Auto-Scaled Chromatogram



Reported by User: Henald John (John)
Report Method: Apex Summary Report
Report Method ID: 1264
Page: 1 of 2

Project Name: INDAPAMIDE TABLET
Date Printed:
1/10/2012
3:16:45 PM Asia/Calcutta

Component Summary Table
Name: Amlodipine

	Sample Name	Name	RT	Area
1	Assay preparation -1	Amlodipine	8.774	4874313
2	Assay preparation -1	Amlodipine	8.756	4861189
Mean			8.765	4867750.850
Std. Dev.			0.013	9279.983
% RSD			0.1	0.2

Component Summary Table
Name: Indapamide

	Sample Name	Name	RT	Area
1	Assay preparation -1	Indapamide	6.626	2097231
2	Assay preparation -1	Indapamide	6.615	2099955
Mean			6.620	2098592.913
Std. Dev.			0.007	1925.742
% RSD			0.1	0.1

FIGURE-22

CHROMATOGRAM FOR ANALYSIS OF FORMULATION (NATRILAM)

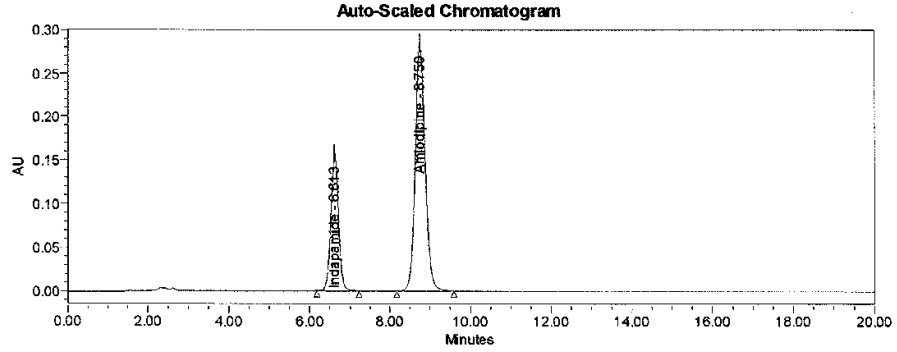
REPEATABILITY - 2

Empower2 **apex** Apex Summary Report

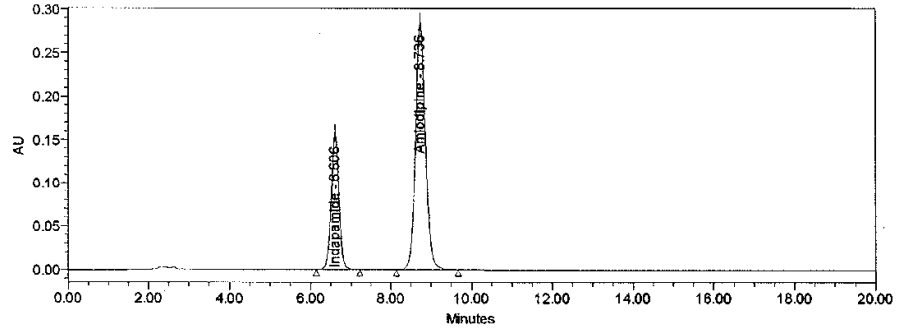
SAMPLE INFORMATION

Sample Name:	Assay preparation -2	Acquired By:	John
Sample Type:	Standard	Sample Set Name:	AMLO_INDAPAMIDE TABS
Vial:	6	Acq. Method Set:	INDAPAMIDE _MSET
Injection #:	1. 2	Processing Method:	INDAPAMIDE AMLO PM
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	20.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 240nm
Date Acquired:	1/9/2012 7:46:08 PM IST, 1/9/2012 8:06:51 PM IST		
Date Processed:	1/10/2012 2:51:56 PM IST, 1/10/2012 2:52:07 PM IST		

Auto-Scaled Chromatogram



Auto-Scaled Chromatogram



Reported by User: Henald John (John)
Report Method: Apex Summary Report
Report Method ID: 1264
Page: 1 of 2

Project Name: INDAPAMIDE TABLET
Date Printed:
1/10/2012
3:17:07 PM Asia/Calcutta

Component Summary Table
Name: Amlodipine

	Sample Name	Name	RT	Area
1	Assay preparation -2	Amlodipine	8.750	4850096
2	Assay preparation -2	Amlodipine	8.736	4854910
Mean			8.743	4852502.718
Std. Dev.			0.010	3403.890
% RSD			0.1	0.1

Component Summary Table
Name: Indapamide

	Sample Name	Name	RT	Area
1	Assay preparation -2	Indapamide	6.613	2098657
2	Assay preparation -2	Indapamide	6.606	2097487
Mean			6.609	2098072.127
Std. Dev.			0.005	826.899
% RSD			0.1	0.0

FIGURE-23

CHROMATOGRAM FOR ANALYSIS OF FORMULATION (NATRILAM)

REPEATABILITY - 3

Empower 2

apex

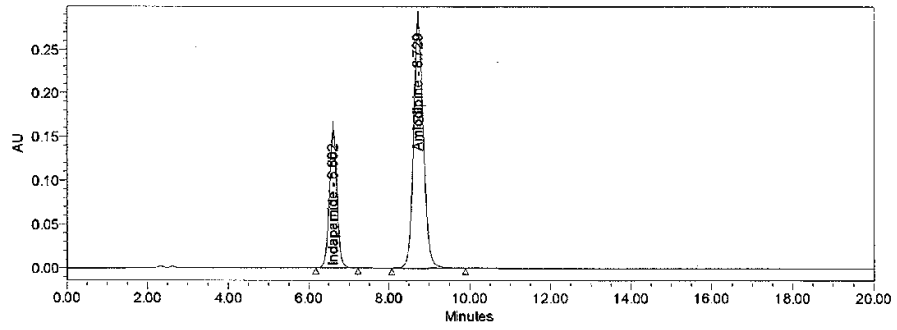
Apex Summary Report

SAMPLE INFORMATION

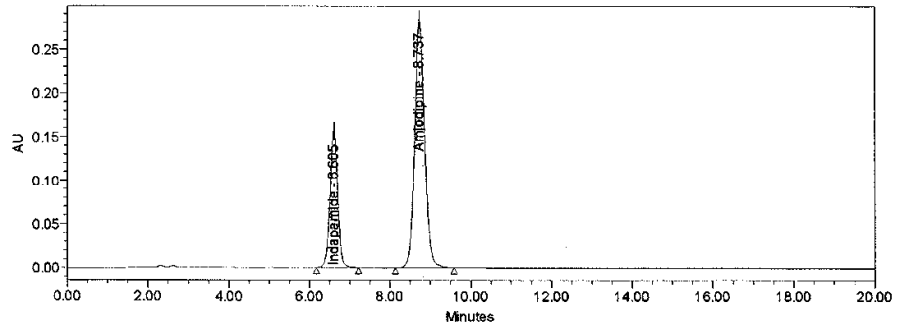
Sample Name:	Assay preparation -3	Acquired By:	John
Sample Type:	Standard	Sample Set Name:	AMLO_INDAPAMIDE TABS
Vial:	7	Acq. Method Set:	INDAPAMIDE _MSET
Injection #:	1, 2	Processing Method:	INDAPAMIDE AMLO PM
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	20.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 240nm

Date Acquired: 1/9/2012 8:27:39 PM IST, 1/9/2012 8:48:28 PM IST
Date Processed: 1/10/2012 2:52:30 PM IST, 1/10/2012 2:52:37 PM IST

Auto-Scaled Chromatogram



Auto-Scaled Chromatogram



Reported by User: Henald John (John)
Report Method: Apex Summary Report
Report Method ID: 1264
Page: 1 of 2

Project Name: INDAPAMIDE TABLET
Date Printed:
1/10/2012
3:18:22 PM Asia/Calcutta

Component Summary Table
Name: Amlodipine

	Sample Name	Name	RT	Area
1	Assay preparation -3	Amlodipine	8.729	4833527
2	Assay preparation -3	Amlodipine	8.737	4825018
Mean			8.733	4829272.247
Std. Dev.			0.006	6016.951
% RSD			0.1	0.1

Component Summary Table
Name: Indapamide

	Sample Name	Name	RT	Area
1	Assay preparation -3	Indapamide	6.602	2081857
2	Assay preparation -3	Indapamide	6.605	2084786
Mean			6.604	2083321.497
Std. Dev.			0.003	2070.761
% RSD			0.0	0.1

FIGURE-24

CHROMATOGRAM FOR ANALYSIS OF FORMULATION (NATRILAM)

REPEATABILITY – 4

Empower 2

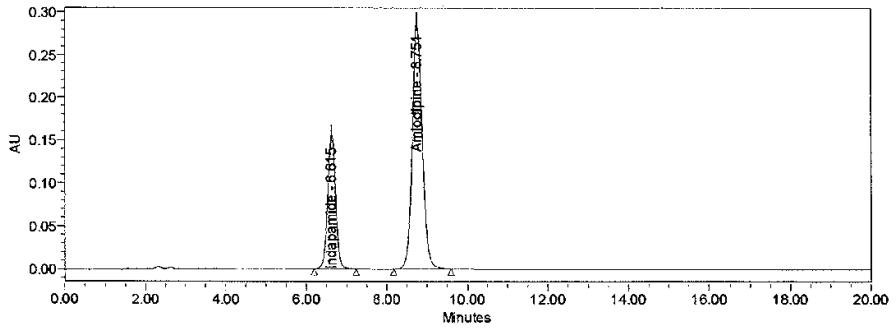
apex

Apex Summary Report

SAMPLE INFORMATION

Sample Name:	Assay preparation -4	Acquired By:	John
Sample Type:	Standard	Sample Set Name:	AMLO_INDAPAMIDE TABS
Vial:	8	Acq. Method Set:	INDAPAMIDE _MSET
Injection #:	1. 2	Processing Method:	INDAPAMIDE AMLO PM
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	20.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 240nm
Date Acquired:	1/9/2012 9:09:13 PM IST, 1/9/2012 9:30:01 PM IST		
Date Processed:	1/10/2012 2:52:51 PM IST, 1/10/2012 2:52:57 PM IST		

Auto-Scaled Chromatogram



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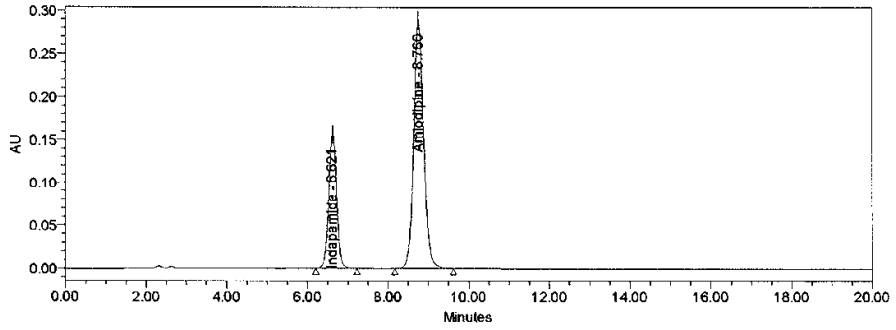


Reported by User: Henald John (John)
Report Method: Apex Summary Report
Report Method ID: 1264
Page: 1 of 2

Project Name: INDAPAMIDE TABLET
Date Printed:
1/10/2012
3:18:44 PM Asia/Calcutta

Component Summary Table
Name: Amlodipine

	Sample Name	Name	RT	Area
1	Assay preparation -4	Amlodipine	8.751	4938144
2	Assay preparation -4	Amlodipine	8.760	4935552
Mean			8.756	4936847.865
Std. Dev.			0.006	1832.734
% RSD			0.1	0.0

Component Summary Table
Name: Indapamide

	Sample Name	Name	RT	Area
1	Assay preparation -4	Indapamide	6.615	2089838
2	Assay preparation -4	Indapamide	6.621	2086270
Mean			6.618	2088053.904
Std. Dev.			0.004	2523.116
% RSD			0.1	0.1

FIGURE-25

CHROMATOGRAM FOR ANALYSIS OF FORMULATION (NATRILAM)

REPEATABILITY – 5

Empower 2

apex

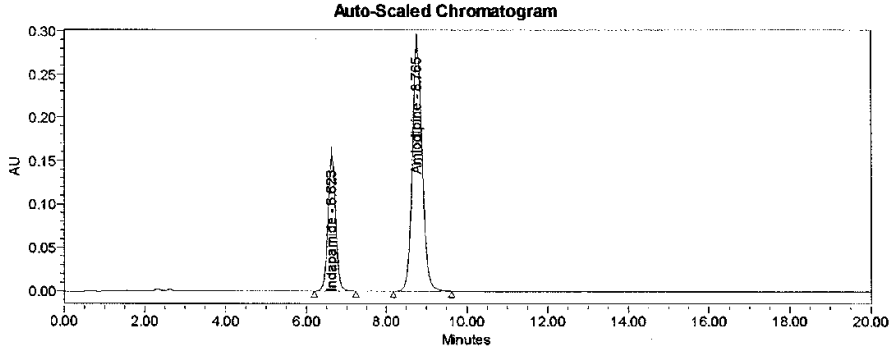
Apex Summary Report

SAMPLE INFORMATION

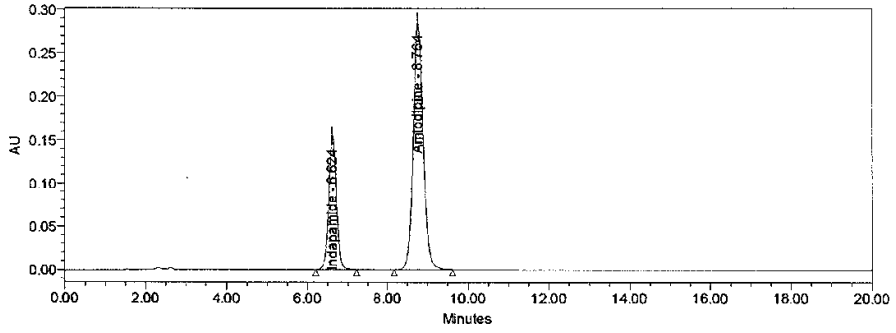
Sample Name:	Assay preparation -5	Acquired By:	John
Sample Type:	Standard	Sample Set Name:	AMLO_INDAPAMIDE TABS
Vial:	9	Acq. Method Set:	INDAPAMIDE _MSET
Injection #:	1, 2	Processing Method:	INDAPAMIDE AMLO PM
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	20.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 240nm

Date Acquired: 1/9/2012 9:50:47 PM IST, 1/9/2012 10:11:30 PM IST
Date Processed: 1/10/2012 2:53:12 PM IST, 1/10/2012 2:53:18 PM IST

Auto-Scaled Chromatogram



Auto-Scaled Chromatogram



Reported by User: Henald John (John)
Report Method: Apex Summary Report
Report Method ID: 1264
Page: 1 of 2

Project Name: INDAPAMIDE TABLET
Date Printed:
1/10/2012
3:18:59 PM Asia/Calcutta

Component Summary Table
Name: Amlodipine

	Sample Name	Name	RT	Area
1	Assay preparation -5	Amlodipine	8.765	4899700
2	Assay preparation -5	Amlodipine	8.764	4895389
Mean			8.764	4897544.167
Std. Dev.			0.001	3048.493
% RSD			0.0	0.1

Component Summary Table
Name: Indapamide

	Sample Name	Name	RT	Area
1	Assay preparation -5	Indapamide	6.623	2072335
2	Assay preparation -5	Indapamide	6.624	2069878
Mean			6.623	2071106.680
Std. Dev.			0.001	1736.946
% RSD			0.0	0.1

FIGURE-26

CHROMATOGRAM FOR ANALYSIS OF FORMULATION (NATRILAM)

REPEATABILITY - 6

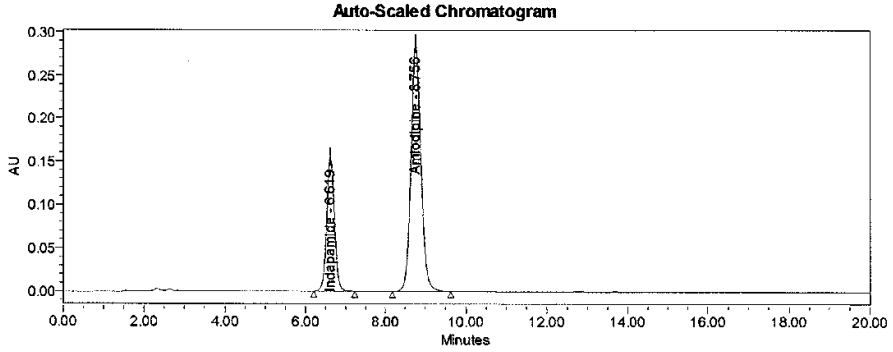
Empower 2 **apex** Apex Summary Report

SAMPLE INFORMATION

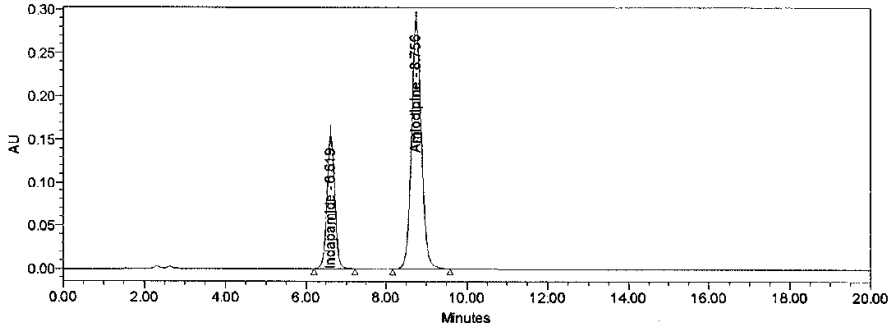
Sample Name:	Assay preparation -6	Acquired By:	John
Sample Type:	Standard	Sample Set Name:	AMLO_INDAPAMIDE TABS
Vial:	10	Acq. Method Set:	INDAPAMIDE _MSET
Injection #:	1. 2	Processing Method:	INDAPAMIDE AMLO PM
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	20.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 240nm

Date Acquired: 1/9/2012 10:32:17 PM IST, 1/9/2012 10:53:05 PM IST
Date Processed: 1/10/2012 2:53:35 PM IST, 1/10/2012 2:53:42 PM IST

Auto-Scaled Chromatogram



Auto-Scaled Chromatogram



Reported by User: Henald John (John)
Report Method: Apex Summary Report
Report Method ID: 1264
Page: 1 of 2

Project Name: INDAPAMIDE TABLET
Date Printed:
1/10/2012
3:20:19 PM Asia/Calcutta

Component Summary Table
Name: Amlodipine

	Sample Name	Name	RT	Area
1	Assay preparation -6	Amlodipine	8.756	4918669
2	Assay preparation -6	Amlodipine	8.756	4915587
Mean			8.756	4917128.131
Std. Dev.			0.000	2179.715
% RSD			0.0	0.0

Component Summary Table
Name: Indapamide

	Sample Name	Name	RT	Area
1	Assay preparation -6	Indapamide	6.619	2080412
2	Assay preparation -6	Indapamide	6.619	2080673
Mean			6.619	2080542.467
Std. Dev.			0.000	183.959
% RSD			0.0	0.0

FIGURE-27

CHROMATOGRAM FOR RECOVERY STUDIES (80%)

Empower2

apex

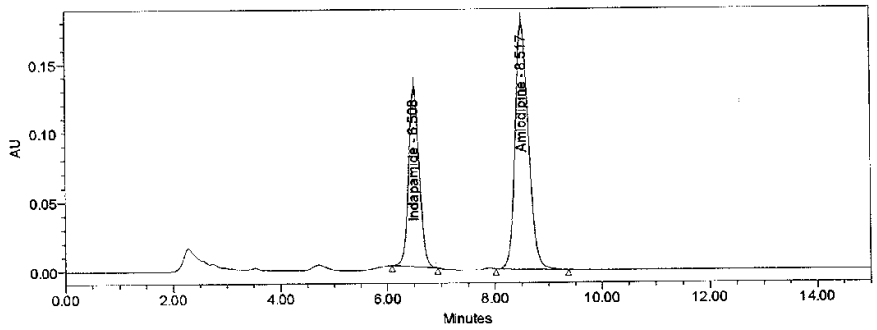
Apex Summary Report

SAMPLE INFORMATION

Sample Name:	Recovery 80%	Acquired By:	John
Sample Type:	Standard	Sample Set Name:	AMLO INDAPAMIDE TABS 12 01
Vial:	3	Acq. Method Set:	INDAPAMIDE _MSET
Injection #:	1, 2, 3	Processing Method:	INDAPAMIDE AMLO PM
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	15.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 240nm

Date Acquired: 1/12/2012 4:43:59 PM IST, 1/12/2012 4:59:44 PM IST, 1/12/2012 5:15:26 PM IST
Date Processed: 1/13/2012 9:29:47 AM IST, 1/13/2012 9:30:10 AM IST, 1/13/2012 9:30:29 AM IST

Auto-Scaled Chromatogram



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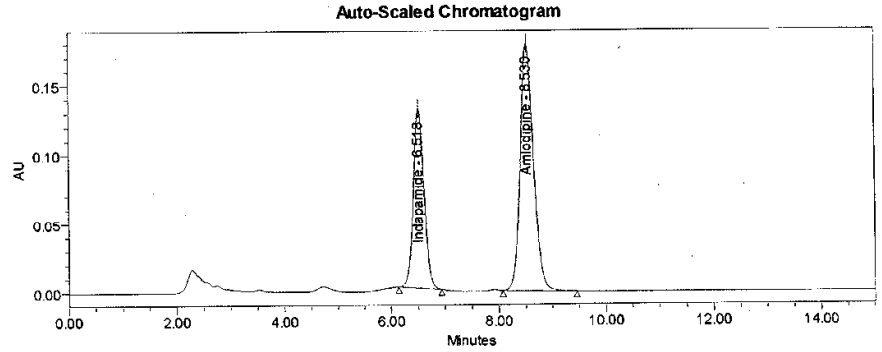
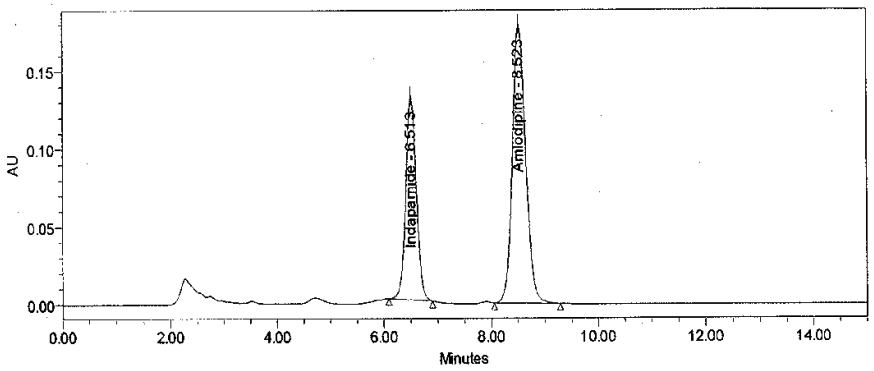


Reported by User: Henald John (John)
Report Method: Apex Summary Report
Report Method ID: 1649
Page: 1 of 2

Project Name: INDAPAMIDE TABLET
Date Printed:
1/13/2012
9:37:13 AM Asia/Calcutta

Auto-Scaled Chromatogram



Component Summary Table
Name: Amlodipine

	Sample Name	Name	RT	Area	USP Plate Count	USP Resolution	USP Tailing
1	Recovery 80%	Amlodipine	8.517	3072827	5810.14	4.90	1.07
2	Recovery 80%	Amlodipine	8.530	3083012	5806.81	4.89	1.07
3	Recovery 80%	Amlodipine	8.523	3079904	5803.77	4.90	1.06
Mean			8.523	3078580.816	5806.9	4.9	1.1
Std. Dev.			0.006	5219.469			
% RSD			0.1	0.2			

Component Summary Table
Name: Indapamide

	Sample Name	Name	RT	Area	USP Plate Count	USP Resolution	USP Tailing
1	Recovery 80%	Indapamide	6.508	1790460	5277.99		1.02
2	Recovery 80%	Indapamide	6.518	1786129	5260.70		1.02
3	Recovery 80%	Indapamide	6.513	1788616	5317.22		1.01
Mean			6.513	1788401.647	5285.3		1.0
Std. Dev.			0.005	2173.208			
% RSD			0.1	0.1			

FIGURE-28

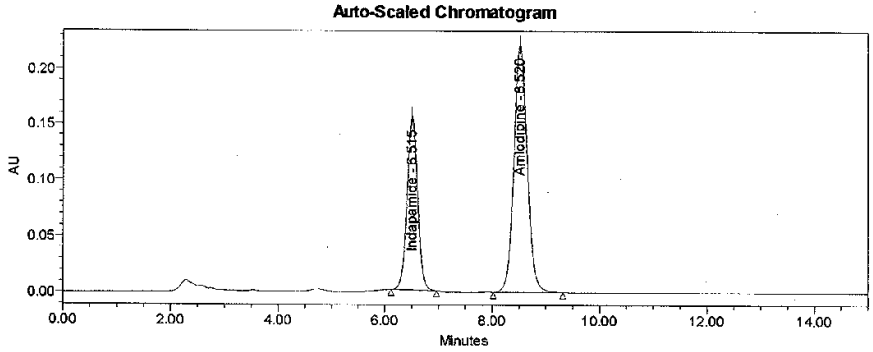
CHROMATOGRAM FOR RECOVERY STUDIES (100%)

Empower2 **apex** Apex Summary Report

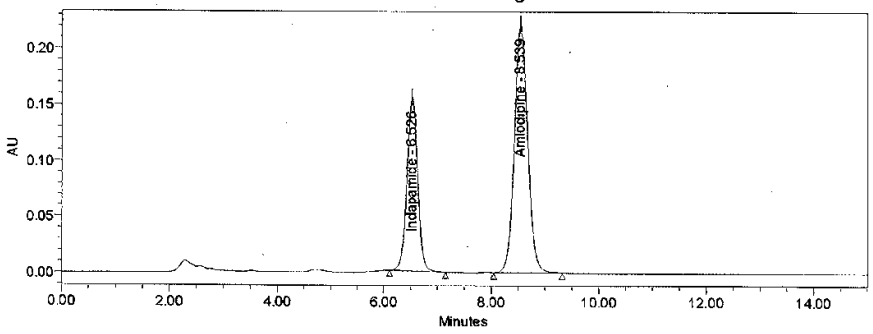
SAMPLE INFORMATION

Sample Name:	Recovery 100%	Acquired By:	John
Sample Type:	Standard	Sample Set Name:	AMLO INDAPAMIDE TABS 12 01
Vial:	4	Acq. Method Set:	INDAPAMIDE _MSET
Injection #:	1, 2, 3	Processing Method:	INDAPAMIDE AMLO PM
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	15.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 240nm
Date Acquired:	1/12/2012 5:31:12 PM IST, 1/12/2012 5:46:58 PM IST, 1/12/2012 6:02:40 PM IST		
Date Processed:	1/13/2012 9:31:01 AM IST, 1/13/2012 9:31:14 AM IST, 1/13/2012 9:31:30 AM IST		

Auto-Scaled Chromatogram



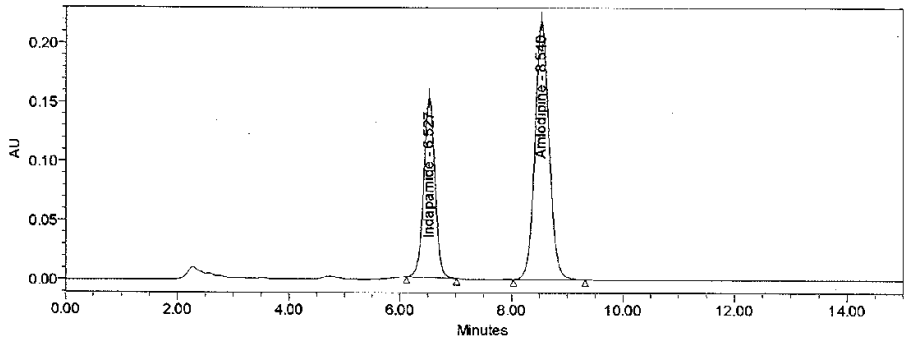
Auto-Scaled Chromatogram



Reported by User: Henald John (John)
Report Method: Apex Summary Report
Report Method ID: 1649
Page: 1 of 2

Project Name: INDAPAMIDE TABLET
Date Printed:
1/13/2012
9:38:37 AM Asia/Calcutta

Auto-Scaled Chromatogram



Component Summary Table
Name: Amlodipine

	Sample Name	Name	RT	Area	USP Plate Count	USP Resolution	USP Tailing
1	Recovery 100%	Amlodipine	8.520	3835064	5742.65	4.87	1.07
2	Recovery 100%	Amlodipine	8.539	3843226	5716.82	4.85	1.07
3	Recovery 100%	Amlodipine	8.540	3849522	5558.34	4.77	1.06
Mean			8.533	3842604.016	5672.6	4.8	1.1
Std. Dev.			0.011	7249.090			
% RSD			0.1	0.2			

Component Summary Table
Name: Indapamide

	Sample Name	Name	RT	Area	USP Plate Count	USP Resolution	USP Tailing
1	Recovery 100%	Indapamide	6.515	2133181	5262.58		1.02
2	Recovery 100%	Indapamide	6.526	2160726	5223.11		1.02
3	Recovery 100%	Indapamide	6.527	2150648	5014.07		1.01
Mean			6.523	2148185.066	5166.6		1.0
Std. Dev.			0.007	13936.613			
% RSD			0.1	0.6			

FIGURE-29

CHROMATOGRAM FOR RECOVERY STUDIES (120%)

Empower 2

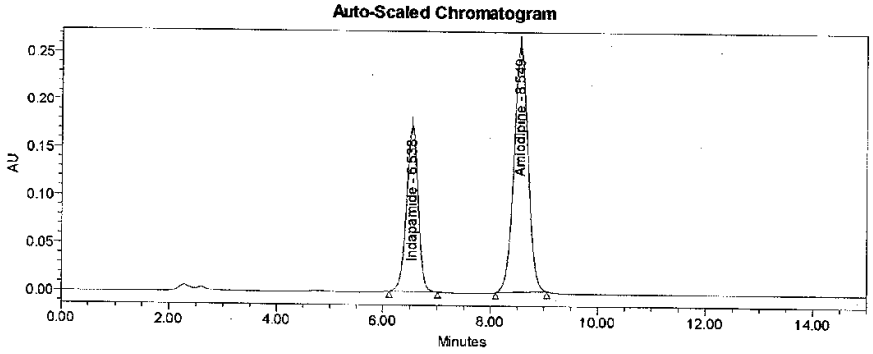
apex

Apex Summary Report

SAMPLE INFORMATION

Sample Name:	Recovery 120%	Acquired By:	John
Sample Type:	Standard	Sample Set Name:	AMLO INDAPAMIDE TABS 12 01
Vial:	5	Acq. Method Set:	INDAPAMIDE _MSET
Injection #:	1, 2, 3	Processing Method:	INDAPAMIDE AMLO PM
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	15.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 240nm
Date Acquired:	1/12/2012 6:18:26 PM IST, 1/12/2012 6:34:11 PM IST, 1/12/2012 6:49:56 PM IST		
Date Processed:	1/13/2012 9:32:39 AM IST, 1/13/2012 9:33:10 AM IST, 1/13/2012 9:33:36 AM IST		

Auto-Scaled Chromatogram



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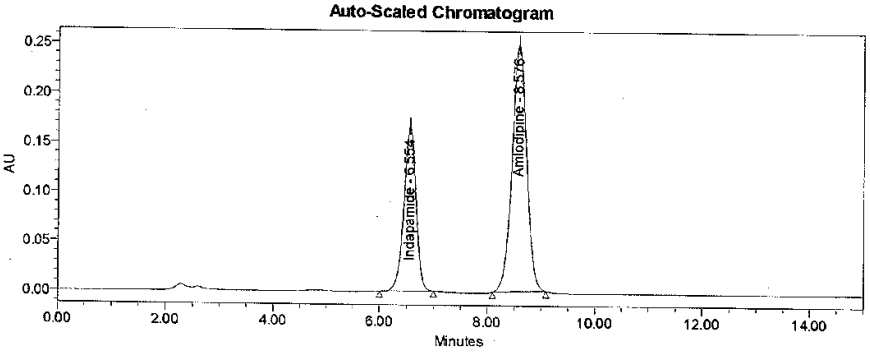
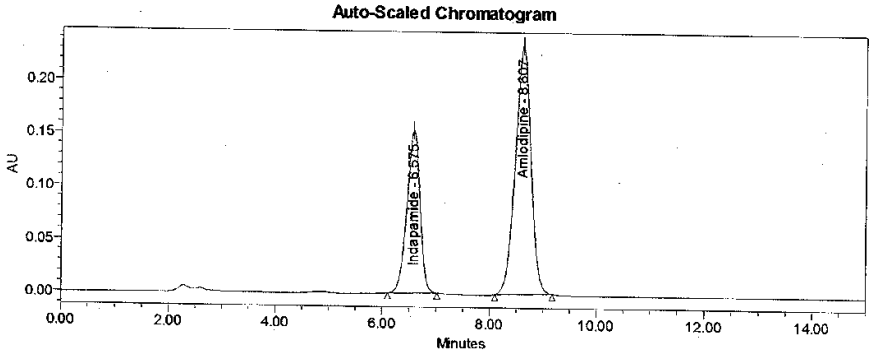


Reported by User: Henald John (John)
Report Method: Apex Summary Report
Report Method ID: 1649
Page: 1 of 2

Project Name: INDAPAMIDE TABLET
Date Printed:
1/13/2012
9:39:37 AM Asia/Calcutta

Auto-Scaled Chromatogram



Component Summary Table
Name: Amlodipine

	Sample Name	Name	RT	Area	USP Plate Count	USP Resolution	USP Tailing
1	Recovery 120%	Amlodipine	8.549	4709741	5133.21	4.56	1.05
2	Recovery 120%	Amlodipine	8.576	4720646	4799.57	4.41	1.02
3	Recovery 120%	Amlodipine	8.607	4759012	4139.50	4.06	1.00
Mean			8.577	4729799.443	4690.8	4.3	1.0
Std. Dev.			0.029	25879.715			
% RSD			0.3	0.5			

Component Summary Table
Name: Indapamide

	Sample Name	Name	RT	Area	USP Plate Count	USP Resolution	USP Tailing
1	Recovery 120%	Indapamide	6.538	2576781	4550.59		0.99
2	Recovery 120%	Indapamide	6.554	2582619	4225.93		0.96
3	Recovery 120%	Indapamide	6.575	2581354	3488.25		0.94
Mean			6.556	2580251.088	4088.3		1.0
Std. Dev.			0.019	3071.367			
% RSD			0.3	0.1			

TABLES

TABLE-1

SOLUBILITY DATA FOR AMLODIPINE BEYSLATE IN POLAR AND NON POLAR SOLVENTS

S.NO	SOLVENT	SOLUBILITY	SOLUBILITY STATUS
1.	Acetone	10 mg in 30 µl	Freely soluble
2.	Acetonitrile	10 mg in 1200 µl	Slightly soluble
3.	Benzene	More than 100 ml	Practically insoluble
4.	Carbon tetra Chloride	10 mg in 10 ml	Practically insoluble
5.	Chloroform	10 mg in 10 µl	Freely soluble
6.	Dichloro methane	10 mg in 20 µl	Freely soluble
7.	Diethyl ether	10 mg in 3000 µl	Slightly soluble
8.	Dimethyl formamide	10 mg in 50 µl	Freely soluble
9.	Distilled water	10 mg in 8000 µl	Slightly soluble
10.	Ethanol	10 mg in 10 µl	Freely soluble
11.	Ethyl Acetate	10 mg in 20 µl	Freely soluble
12.	Glacial acetic acid	10 mg in 20 µl	Freely soluble
13.	Hexane	More than 100 ml	Practically insoluble
14.	Hydrochloric acid (0.1 M)	10 mg in 20 ml	Very slightly soluble
15.	Isopropyl alcohol	10 mg in 5000 µl	Slightly soluble
16.	Methanol	10 mg in 15 µl	Freely soluble
17.	n-butanol	10 mg in 1300 µl	Slightly soluble
18.	Petroleum ether	More than 100 ml	Practically insoluble
19.	Sodium hydroxide (0.1 M)	More than 100 ml	Practically insoluble
20.	Toluene	More than 100 ml	Practically insoluble

TABLE-2

SOLUBILITY DATA FOR INDAPAMIDE IN POLAR AND NON POLAR SOLVENTS

S.NO	SOLVENT	SOLUBILITY	SOLUBILITY STATUS
1.	Acetone	10 mg in 10 µl	Freely soluble
2.	Acetonitrile	10 mg in 30 µl	Freely soluble
3.	Benzene	More than 100 ml	Practically insoluble
4.	Carbon tetra Chloride	10 mg in 10 ml	Practically insoluble
5.	Chloroform	10 mg in 3 ml	Slightly Soluble
6.	Dichloro methane	10 mg in 10 ml	Slightly soluble
7.	Diethyl ether	10 mg in 10 ml	Slightly soluble
8.	Dimethyl formamide	10 mg in 30 µl	Freely soluble
9.	Distilled water	More than 100 ml	Practically insoluble
10.	Ethanol	10 mg in 90 µl	Freely Soluble
11.	Ethyl Acetate	10 mg in 100 µl	Soluble
12.	Glacial acetic acid	10 mg in 90 µl	Freely soluble
13.	Hexane	More than 100 ml	Practically insoluble
14.	Hydrochloric acid (0.1 M)	10 mg in 3000 µl	Slightly insoluble
15.	Isopropyl alcohol	10 mg in 3000 µl	Slightly soluble
16.	Methanol	10 mg in 30 µl	Freely soluble
17.	n-butanol	10 mg in 600 µl	Soluble
18.	Petroleum ether	More than 100 ml	Practically insoluble
19.	Sodium hydroxide (0.1 M)	10 mg in 1000 µl	Sparingly soluble
20.	Toluene	More than 100 ml	Practically insoluble

TABLE-3

OPTICAL CHARACTERSTICS OF AMLODIPINE BESYLATE AND INDAPAMIDE BY FIRST ORDER DERIVATIVE SPECTROSCOPIC METHOD

PARAMETERS	AMLODIPINE BESYLATE AT 339.0 nm*	INDAPAMIDE AT 293.0 nm*
Beers law limit (µg mL^{-1})	15-90 (µg mL^{-1})	5-30 (µg mL^{-1})
Molar absorptivity	176.6787	236.2281
Sandells sensitivity (µg/cm^2/0.001 A.U)	3.032491	1.590909
Correlation coefficient (r)	0.999842	0.999876
Regression equation (y= mx + c)	Y = 0.0033x - (-)0.00018	Y = 0.0006 x 0.000171
Slope (m)	0.00033	0.000629
Intercept (c)	-0.00018	0.000171
LOD (µg mL^{-1})	0.3973	0.2177
LOQ (µg mL^{-1})	1.2041	0.6597

*Mean of six observations

TABLE-4

QUANTIFICATION OF TABLET FORMULATION (NATRILAM) BY FIRST ORDER DERIVATIVE SPECTROSCOPIC METHOD

Drug	Sample No.	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained*	Average (%)	S.D	% R.S.D.	S.E.
AML	1	5	4.91	98.20	98.60	0.61967	0.6247	0.0172
	2		4.97	99.40				
	3		4.97	99.40				
	4		4.91	98.20				
	5		4.91	98.20				
	6		4.91	98.20				
IND	1	1.5	1.49	99.33	99.325	1.25544	1.26398	0.0348
	2		1.46	97.33				
	3		1.49	99.33				
	4		1.52	101.3				
	5		1.49	99.33				
	6		1.49	99.33				

*Mean of six observations

TABLE -5

INTRA DAY AND INTER DAY ANALYSIS OF FORMULATION BY FIRST ORDER DERIVATIVE SPECTROSCOPIC METHOD

Drug	Sample No.	Labeled amount (mg/tab)	Percentage obtained*		S.D		% R.S.D.	
			Intra day	Inter day	Intra day	Inter day	Intra day	Inter day
AML	1	5	98.20	98.20	0.69282	0.11547	0.7026	0.11751
	2	5	98.20	98.40				
	3	5	98.40	98.20				
Mean			98.26	98.26				
IND	1	1.5	99.33	99.33	1.15470	1.1547	1.1703	1.17830
	2	1.5	97.33	97.33				
	3	1.5	99.33	97.33				
MEAN			98.66	97.99				

*Mean of Three observations

TABLE -6

RUGGEDNESS STUDY BY DERIVATIVE SPECTROSCOPIC METHOD

Drug	Condition	Average* % Obtained	S.D	% R.S.D	S.E.
AML	Analyst 1	98.33	0.11547	0.117427	0.01283
	Analyst 2	98.20	0.69282	0.117830	0.07698
	Instrument 1	98.33	0.11547	0.11742	0.01283
IND	Analyst 1	97.96	1.1543701	0.178306	0.1183
	Analyst 2	97.80	1.154701	1.170344	0.1283
	Instrument 1	98.68	1.154701	1.170344	0.1283

*Mean of Three observations

TABLE-7

RECOVERY STUDY DATA OF 50% PRE ANALYSED FORMULATION BY FIRST ORDER DERIVATIVE SPECTROSCOPIC METHOD

Drug	Amount present* ($\mu g\ mL^{-1}$)	Amount Added* ($\mu g\ mL^{-1}$)	Amount estimated* ($\mu g\ mL^{-1}$)	Amount recovered* ($\mu g\ mL^{-1}$)	% Recovery* ($\mu g\ mL^{-1}$)	S.D.	% R.S.D.	S.E.
AML	25	15	39.86	14.86	99.08	0.45061	0.45659	0.01251
	25	22.5	47.15	22.15	98.14			
	25	30	54.49	29.55	98.51			
	25	37.5	61.84	36.83	98.26			
	25	45	69.65	44.65	99.22			
	25	52.5	76.94	51.94	98.94			
IND	7.5	5	12.5	5.00	100.00	0.45296	0.45350	0.01258
	7.5	7.5	14.96	7.47	99.73			
	7.5	10	17.55	10.05	100.56			
	7.5	12.5	19.96	12.46	99.76			
	7.5	15	22.33	14.88	99.24			
	7.5	17.5	24.95	17.46	99.79			

*Mean of Three observations

TABLE-8

OPTICAL CHARACTERISTICS OF AMLODIPINE BESYLATE AND INDAPAMIDE BY RP - HPLC

PARAMETERS	AMLODIPINE*	INDAPAMIDE*
λ_{max} (nm)	240	240
Beers law limit (μg mL^{-1})	80-120	24-36
Correlation coefficient (r)	0.99993	0.99965594
Régression équation (y=mx+c)	Y= (34513.19)x+717.1428	Y=(70554.93)x-(-1552.982143)
Slope (m)	34513.19	70554.93929
Intercept (c)	717.1428	-1552.982143
LOD (μg mL^{-1})	0.07491703	0.066817129
LOQ (μg mL^{-1})	0.227021308	0.202476148
Standard Error	3191.477	8481.610289

*Mean of Three observations

TABLE -9

QUANTIFICATION OF TABLET FORMULATION (NATRILAM)

BY RP-HPLC

Drug	Sample No.	Labeled amount (mg/tab)	Amount found (mg/tab)	Percentage Obtained*	Average (%)	S.D.	% R.S.D.	S.E.
AML	1	5	5.082	101.65	100.86	0.702332	0.696309	0.019509
	2		5.003	100.06				
	3		5.057	101.14				
	4		5.006	100.12				
	5		5.077	101.54				
	6		5.037	100.74				
IND	1	1.5	1.506	100.40	100.61	0.447169	0.444458	0.012421
	2		1.511	100.73				
	3		1.508	100.53				
	4		1.521	101.39				
	5		1.508	100.57				
	6		1.501	100.04				

* Mean of six observations

TABLE-10

RECOVERY STUDIES OF AMLODIIPINE BESYLATE AND INDAPAMIDE

BY RP-HPLC

Drug	Percentage	Amount added* ($\mu g\ mL^{-1}$)	Amount recovered* ($\mu g\ mL^{-1}$)	% Recovery*	S.D.	% R.S.D.	S.E.
AML	80	80	79.70	99.53	0.34219	0.34389	0.03802
	100	100	99.47	99.47	0.15821	0.15905	0.01758
	120	120	122.46	102.04	0.55018	0.39182	0.06113
IND	80	24	24.68	102.55	0.41061	0.39995	0.04555
	100	30	29.63	98.83	0.62601	0.63343	0.06955
	120	36	35.61	99.26	0.61808	0.62226	0.06867

*Mean of Three observations

TABLE-11

RUGGEDNESS STUDY BY RP-HPLC METHOD

Drug	Condition	Average* % Obtained	S.D	% R.S.D	S.E.
AML	Analyst 1	99.82	0.141067	0.141322	0.141322
	Analyst 2	100.60	0.752684	0.74817	0.083682
IND	Analyst 1	101.22	0.563205	0.556417	0.06257
	Analyst 2	102.09	1.25576	1.230012	1.39529

*Mean of Three observations

TABLE - 12

SYSTEM SUITABILITY TEST PARAMETERS FOR THE OPTIMIZED CHROMATOGRAM BY RP - HPLC

PARAMETERS	**AMLODIPINE BESYLATE**	**INDAPAMIDE**	**LIMIT**
Tailing factor	1.25	1.38	≤ 2
Asymmetrical factor	1.36	1.61	≤ 2
Theoretical plates	6080.68	4481	> 2000
Capacity factor	5.97	4.91	> 2.0
Theoretical plate per unit Length	243.22	426.48	----
Resolution	6.0325		≥2

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